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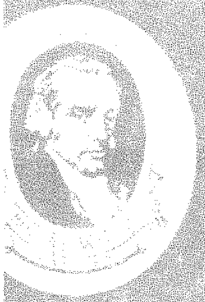

British Journal of Dermatology

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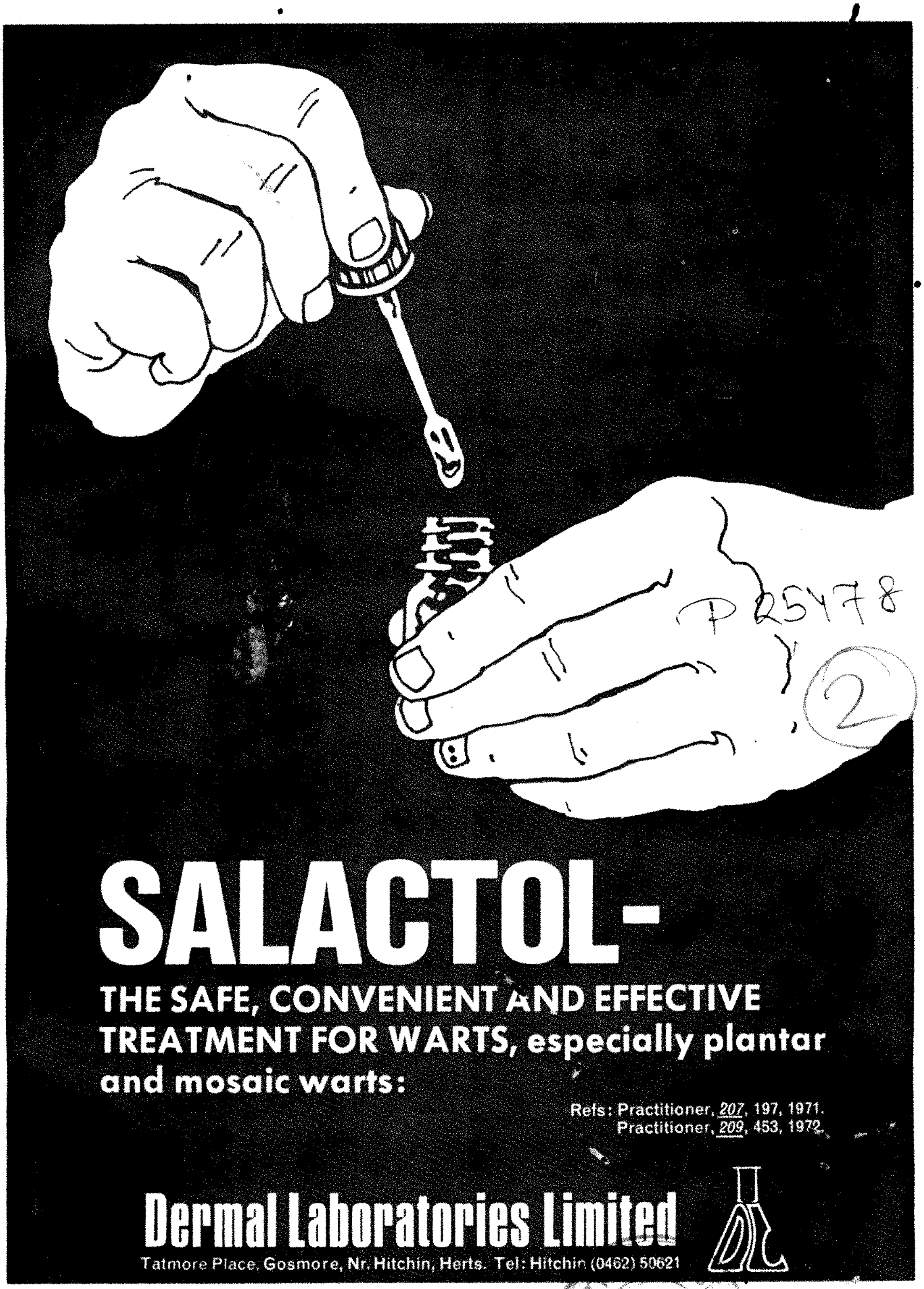

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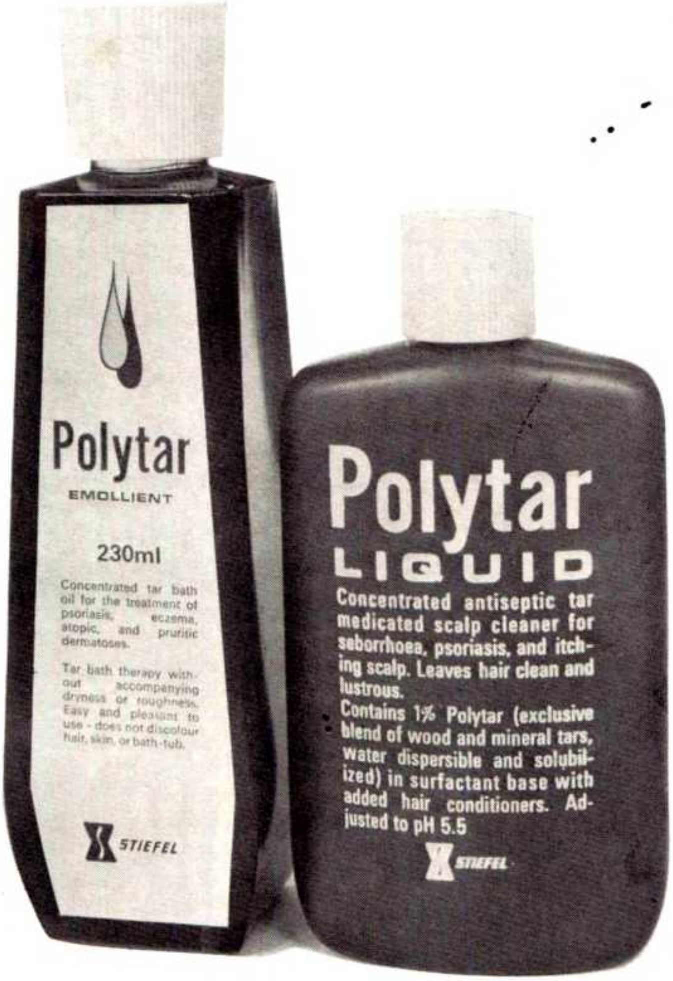
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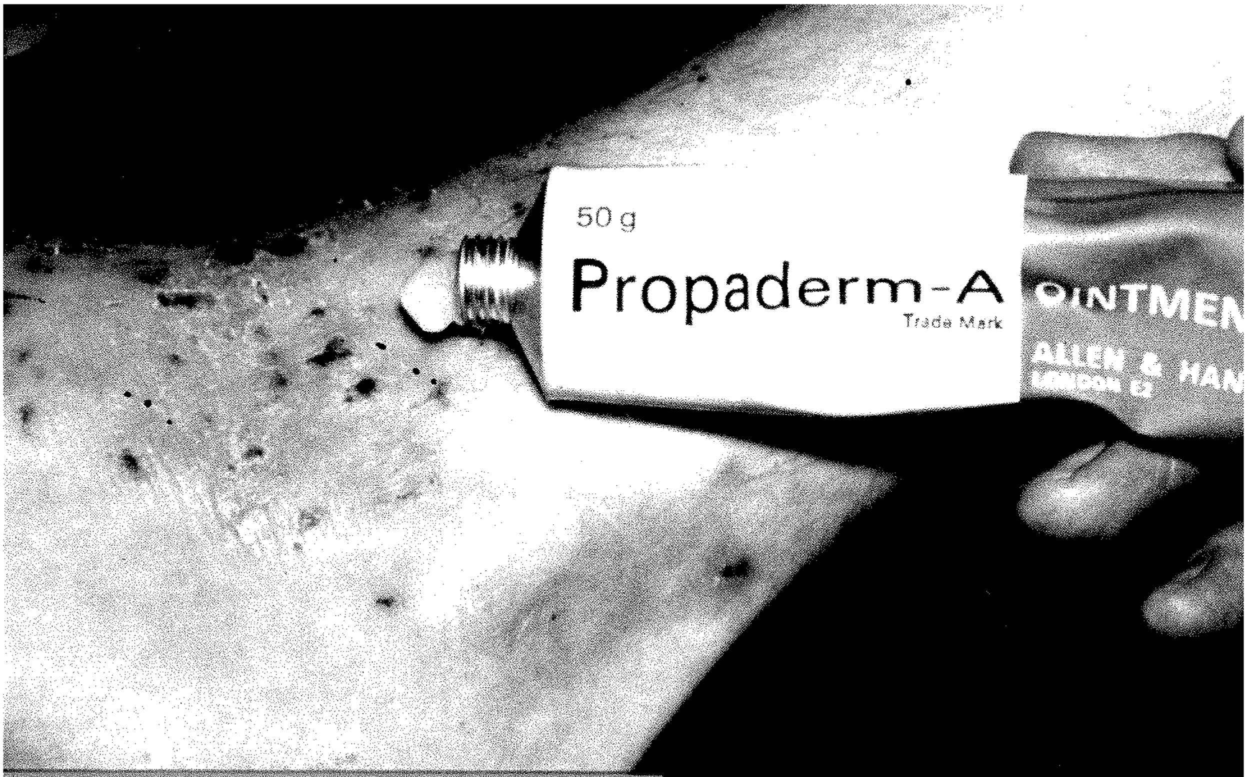

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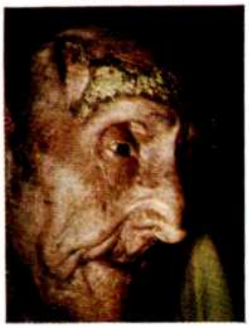

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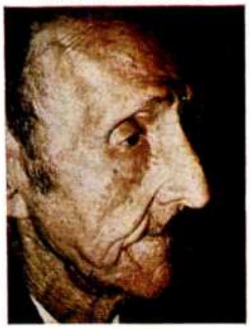

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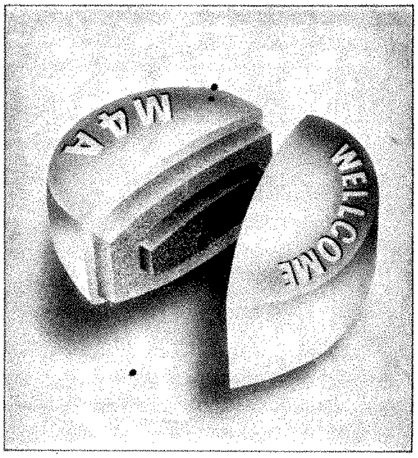

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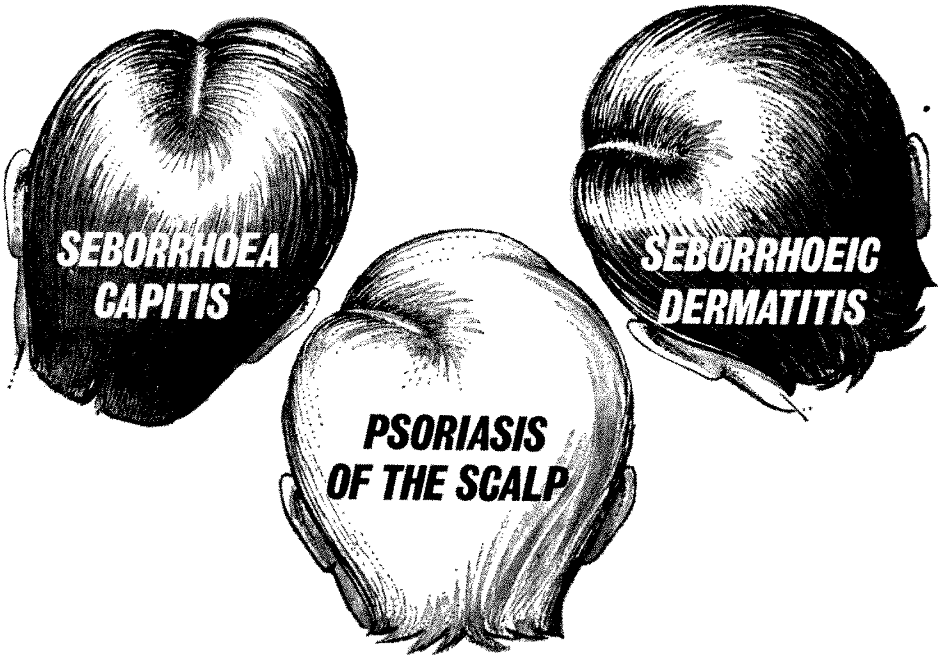

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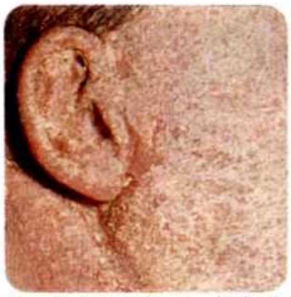
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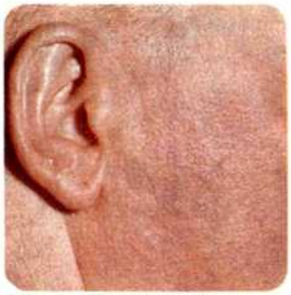
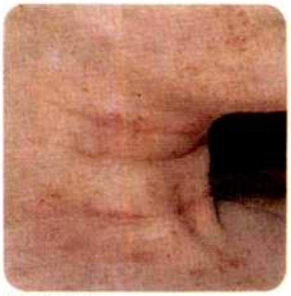
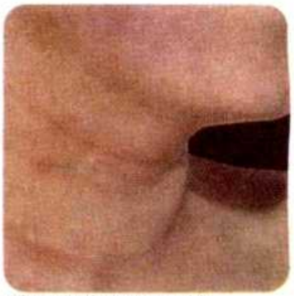
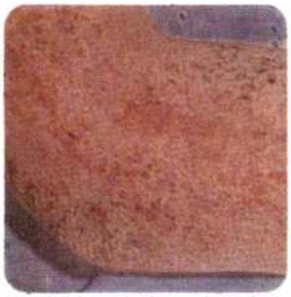
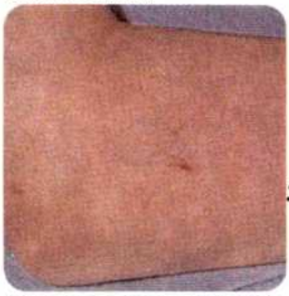

After 7 days' treatment with Betnovate Ointment


Atopic eczema


After 4 days' treatment with Betnovate Ointment


Atopic dermatitis of 3 years' duration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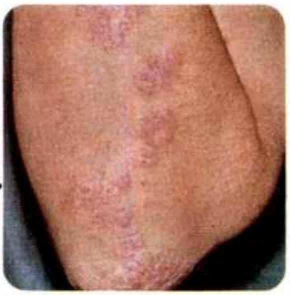
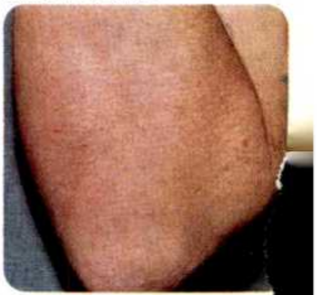
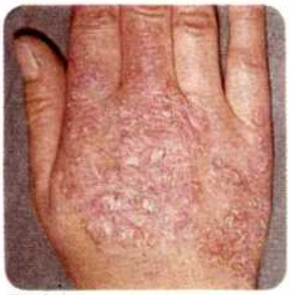
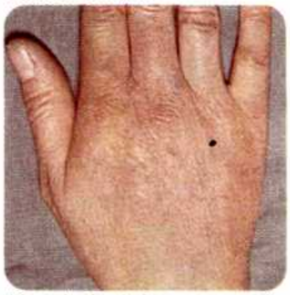
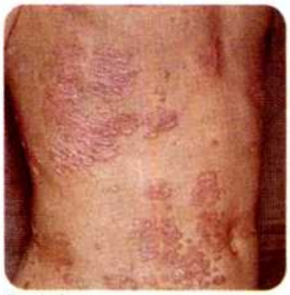
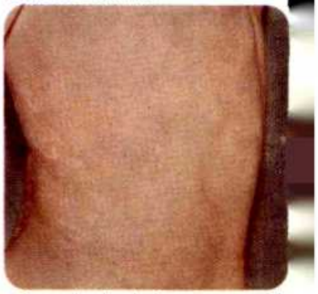

Psoriasis of 20 years' duration

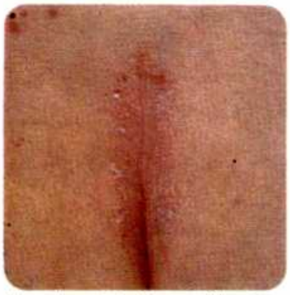

After 12 days' occlusive treatment wit Betnovate Ointment

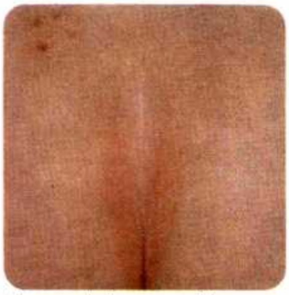

Psoriasis

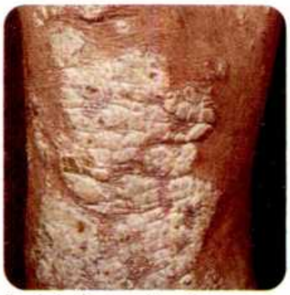

After 9 days' occlusive treatment with Betnovate Ointment

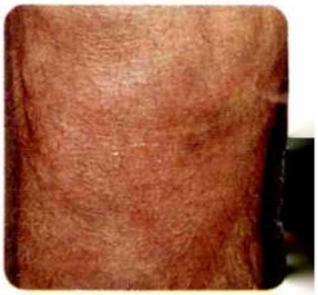

Psoriasis


After 21 days' occlusive treatment with Betnovate


Flexural psoriasis of 15 years' duration


After 4 weeks' treatment with Betnovate Ointment


Psoriasis of 51 years' duration


After 24 days' occlusive treatment with Betnovate Ointment

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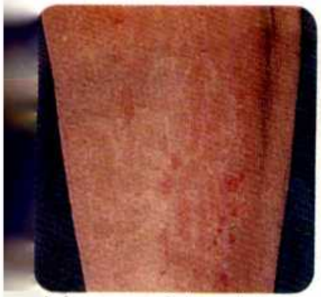
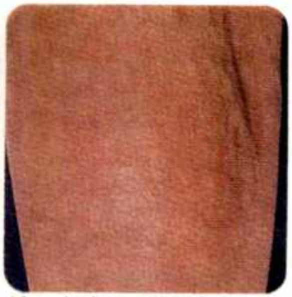
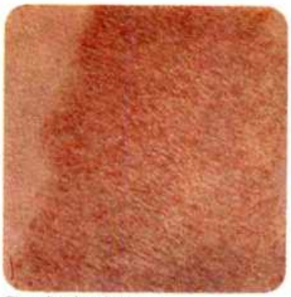
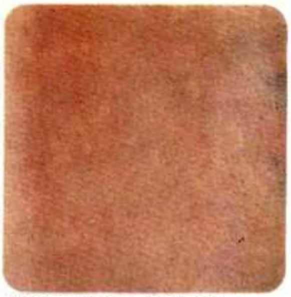
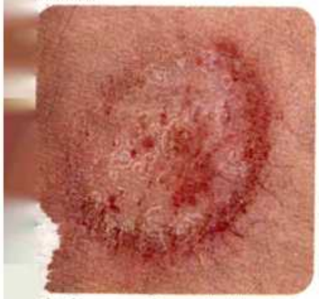
again. And again.

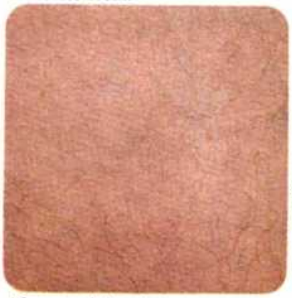

aricose eczema of 4/5 months' uration

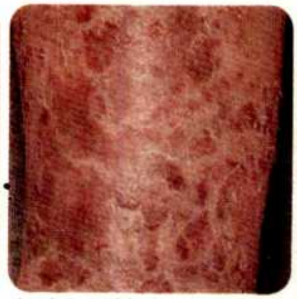

After 7 days' treatment with Betnovate Cream

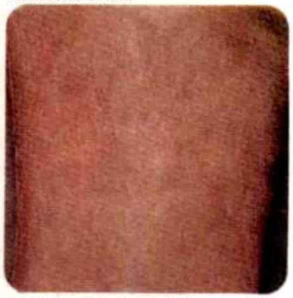

Constitutional eczema


After 4 weeks' treatment with Betnovate Cream


iscoid eczema of 9 months' duration


After 2 weeks' occlusive treatment with Betnovate Ointment


Atopic dermatitis of 12 years' duration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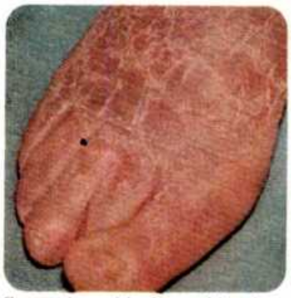
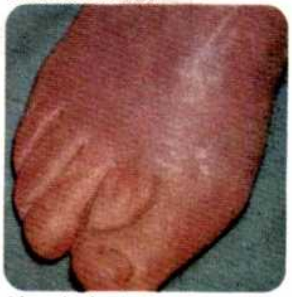
*Lancet (1964) 1, 1,177


Contact dermatitis of 2 years' duration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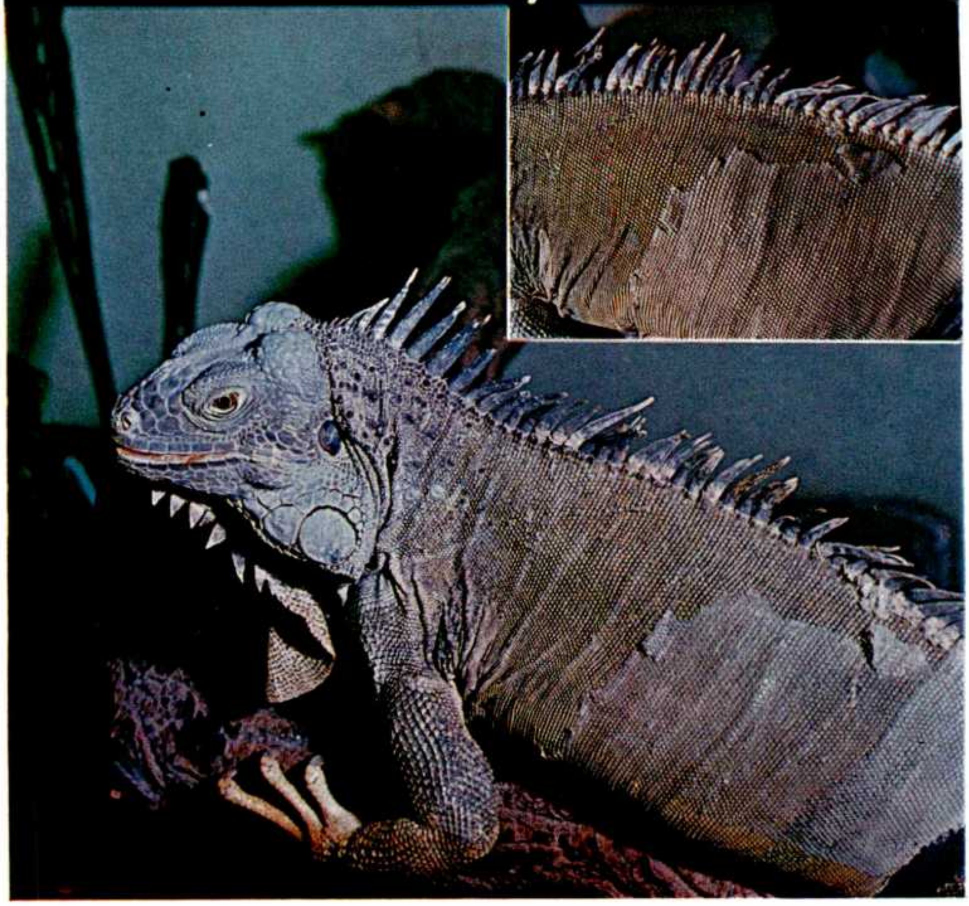

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Clinical and Laboratory Investigations

Anderson–Fabry disease*

H.J.WALLACE

Skin Department, St Thomas's Hospital, London

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SUMMARY

Anderson–Fabry disease (AFD) syn. angiokeratoma corporis diffusum is an uncommon sex-linked disorder of sphingolipid metabolism associated with a deficiency of ceramide trihexosidase and widespread accumulation predominantly of ceramide trihexoside. In males, AFD usually presents in childhood or early adolescence with intermittent and often excruciating pain in fingers and toes. Oedema, initially transient, hypohidrosis and obscure fever are also common. Renal damage is usually detectable in the early twenties and is ultimately the most frequent cause of death, usually in the late thirties or early forties. Cerebrovascular and cardiac disturbances are also common, often with a normal blood pressure. In both sexes a characteristic symptomless corneal dystrophy is usually present by early adult life. The average expectation of life in males is 42 years. In females the disorder may remain symptomless and their expectation of life is seldom impaired.

This paper reports the clinical features in fifty-seven patients with AFD including twelve females. Recent advances in the elucidation of the biochemical abnormality are discussed including leucocyte alphagalactosidase activity in eighteen patients. Histological studies including histochemical and electron microscopy findings are also reported including the first account of AFD in the uterus, enabling an estimate to be made of rate of deposition of abnormal glycolipid in the endometrium.

The subject of this lecture concerns an uncommon sex-linked disorder of sphingolipid metabolism to which many titles have been applied from the grandiloquent and misleading angiokeratoma corporis diffusum, to the historically inaccurate Fabry's disease. With these initial shortcomings and the very brief account to be found even in the largest textbooks of medicine it is not surprising that correct diagnosis is the exception rather than the rule.

In males this disorder usually presents in childhood or early adolescence with intermittent and often excruciating pain in fingers and toes, the characteristic eruption at this stage often being absent or ill-developed. Oedema initially transient, hypohidrosis and obscure fever are also common. Renal damage is usually detectable in the early twenties and is ultimately the most frequent cause of death, usually in the late thirties or early forties. Cerebrovascular and cardiac disturbances are also common, often with a normal blood pressure. In both sexes a characteristic symptomless corneal dystrophy is usually present by early adult life. The average expectation of life in males is 42 years. In females the disorder may remain symptomless and their expectation of life is seldom impaired. I hope to show that this disorder merits the attention of all physicians and that it may well provide valuable clues in the understanding of some aspects of disordered function particularly in the kidney and central nervous system.

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* The Watson-Smith Lecture, delivered before the Royal College of Physicians of London, 1972.

My other reason for drawing attention to this disorder is unashamedly parochial. This erudite audience will doubtless be aware that the first preparation of brain lipids was made by Dr Thudichum, lecturer in pathological chemistry at St Thomas's Hospital from 1865 to 1870. It is, therefore, particularly appropriate that the dyslipidosis which is the subject of my lecture should have been first described by Mr William Anderson, surgeon to St Thomas's Hospital. In an age in which medical education and career structure are in a state of flux I hope that I may be allowed to digress for a few moments to refer briefly to the career of this remarkable, but little known man whose interests ranged so widely. After an early education at the City of London School and the Lambeth School of Art he had a distinguished record as a student at St Thomas's. Following a short period as demonstrator of anatomy and as surgical registrar he was invited to become director of Japanese Naval Medical College in Tokyo, a post which he filled with great distinction. He was particularly successful as a teacher, learning Japanese rapidly so that within a short time he was able to lecture without an interpreter. His artistic talent enabled him to become one of the leading authorities on Japanese pictorial art, his collection forming the basis of Japanese pictures and drawings in the British Museum. Elected to the surgical staff of St Thomas' in 1880 he later became head of the skin department. He combined his medical and artistic interests so successfully that in 1891 he was also elected Professor of Anatomy at the Royal Academy of Arts, continuing to make significant contributions to surgery, dermatology and the arts until his death in 1900.

In 1897 at a meeting of the Dermatological Society of London he demonstrated under the title 'A case of angeokeratoma' (Anderson, 1898) a house painter aged 39 who had been admitted to St Thomas's for a neuroma of the thigh and was noted to have multiple symptomless angiectases. These had begun at the age of 11 in front of the knees, gradually spreading to the trunk and upper extremities over the next 11 years with no spread thereafter. They varied in size from a pinpoint to a hemp seed, the smallest lesions being impalpable. The colour ranged from dark purple to almost black. They were most profuse on the inner side of the thighs and genitalia, absent from the head and neck and sparse over the upper trunk and extremities. Histology revealed varicose angiectases in the papillary layer of the dermis with slight hyperkeratinization. Anderson also noted other abnormalities including proteinuria, varicose veins, deformity of the little finger and leg oedema and suggested that the proteinuria might be related to a widespread disorder of blood vessels.

In 1898 Fabry reported a similar eruption to which he gave the erroneous title of purpura nodularis later correcting this to angiokeratoma but without noting any systemic manifestations. In 1915 in a review of angiokeratoma he classified his patient under the heading of angiokeratoma corporis diffusum, rejecting without giving any reason Anderson's initial contribution (Fabry, 1898, 1915).

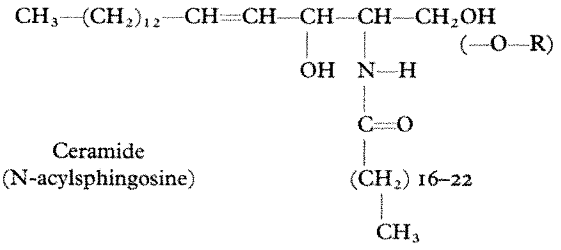
Eponyms are now rightly decried, but until the precise chemical defects in this and similar disorders have been established and have been rendered into intelligible language, eponymous titles are likely to continue. Meanwhile historical accuracy requires that the name of Anderson should take precedence or at least be linked with the name of Fabry as Anderson–Fabry disease (AFD) a belated tribute that has now, happily, found its way into standard books of eponyms. (Kerdel-Vegas & Adamicska, 1967; Jablonska, 1969). At present the title Anderson–Fabry disease (AFD) has, I suspect, little to fear from proposed monstrosities such as gal-gal-cer sphingolipodystrophy or jingles such as ceramide trihexosidosis.

After Anderson's initial observation of albuminuria recognition of the systemic manifestations of this disorder came slowly over the next 40 years, the diagnosis of the fourth recorded patient apparently eluding the great Sir William Osler (Madden, 1912). A further twenty-one patients with a multiplicity of signs and symptoms were recorded up to 1947, including one doctor. A significant advance was made in a publication by Ruiter and his colleagues (Ruiter, Pompen & Wijers, 1947) of the post-mortem findings in two brothers both of whom had died of uraemia. They noted an unusual vacuolation of the smooth muscle of the blood vessels and muscle fibres of the heart, and suggested that these changes might be caused by a storage disorder, later identified as a lipid by Scriba (1950).

THE CHEMICAL DEFECT

Against the remote possibility that there may be a few of my audience who share with me the difficulty of understanding the intricacies of lipid chemistry, I thought that it might be appropriate at an early stage to deal briefly with sphingolipids in relation to this disorder. You will remember that sphingolipids are a class of complex lipids which have in common a long chain unsaturated aliphatic amino-alcohol, sphingosine, as opposed to glycerol, the alcohol of glycerides and glycerophosphatides. The amino group of this alcohol is acylated by one of a number of long chain saturated fatty acids to give rise to a ceramide or an N-acylsphingosine, and thus to the four major groups of the sphingolipids.

TABLE 1. Sphingolipids



$$CH_3-(CH_2)_{12}-CH{=}CH-\underset{\displaystyle OH}{CH}-\underset{\displaystyle N-H-C{=}O-(CH_2)_{16-22}-CH_3}{CH}-CH_2OH\ (-O-R)$$

Ceramide
(N-acylsphingosine)

Sphingolipid	Nature
Sphingomyelin	Ceramide-phosphorylcholine
Cerebroside	Ceramide-glucose Ceramide-galactose
Sulfatide	Ceramide-galactose-3-sulphate
Gangliosides	Ceramide-glucose-galactose (-neuraminic acid)-galactosamine-galactose

Excluding sphingomyelin these lipids are also sometimes known as glycolipids because of their hexose content. Sphingolipodystrophies are characterized by the accumulation in tissues of lipid material.

It is thought that this is due in each instance to a deficiency of a specific enzyme concerned with a particular step in the degradation of an individual sphingolipid. It appears that the parent sphingolipid in AFD is at least closely similar to the major parent lipid in Gaucher's disease, a 'globoside'. According to Brady (1969) there are five steps in the breakdown of this substance to sphingosine.

In AFD the failure is in the second step and perhaps also in the third due to a deficiency of one or possibly more ceramide trihexosidases also known as galactosidases. These are found in several normal tissues including liver, kidney, spleen, small intestine, brain and in blood. Deficiency results in failure to split off the terminal galactose of the trihexoside and possibly dihexoside resulting in accumulation of these materials in several tissues particularly the kidney. These changes are summarized in Table 4.

Regarding the blood, Dr Bacchus working in Professor Prunty's laboratory has estimated the leucocyte alpha-galactosidase activity in eighteen patients with AFD, twelve males and six females. His detailed findings will be published later but in brief there was diminished alpha- but not beta-galactosidase activity in all but one compared with controls. The deficiency was much greater in males than females and was in general proportional to the severity of symptoms and the duration of the disorder. The one patient with normal enzyme activity was a known carrier with characteristic eye changes but free of other features of AFD.

In diagnosis, some biochemical investigation may be useful particularly in childhood and in the detection of carriers. Thus, an increased urinary excretion of ceramide trihexoside has been reported in a newborn boy (Philippart, Sarlieve & Manocorda, 1969). Prenatal diagnosis may well prove to be possible by the estimation of the alpha-galactosidase activity of the epithelial cells and fibroblasts of the amniotic fluid. Some results are of genetic interest. The effect of sex linkage is manifest in female carriers in whom the lowering of leucocyte alpha-galactosidase activity is between 15 and 40% less than in males (Kint, 1970). Again in males, while the urinary excretion of ceramide trihexoside is usually greater than that of ceramide dihexoside, in females, ceramide dihexoside predominates. Studies on alpha-galactosidase activity in cultured fibroblasts also supports the Lyon hypothesis which proposes that in each somatic cell of the female one of the two X chromosomes is genetically inactive

TABLE 2. The sphingolipodystrophies

Lipodystrophy	Accumulated lipid	Enzyme deficiency (and degradative step)
Niemann–Pick disease	Sphingomyelin	Sphingomyelinase (I)
Anderson–Fabry disease	Ceramide-trihexoside	Ceramide trihexosidase (II)
Gaucher's disease	Glucocerebroside	Glucocerebrosidase (IV)
Metachromatic lipodystrophy	Sulphatide	Sulphatidase (I)
Generalized gangliosidosis	Monosialoganglioside	Galactosidase (I)
Tay–Sachs disease	Tay–Sach ganglioside	? (II)

TABLE 3. Degradation of globoside

Globoside	Ceramide-glucose-galactose-galactose-N acetylgalactosamine	
	↓	
Ceramide-trihexoside	Ceramide-glucose-galactose-galactose	
	↓	Anderson–Fabry disease
	Ceramide-glucose-galactose	
	↓	
Glucocerebroside	Ceramide-glucose	
	↓	Gaucher's disease
	Ceramide	
	↓	
	Sphingosine + fatty acid	

and that this inactivity is maintained in its progeny. Romeo & Migeon (1970) have shown the females with AFD have two distinct clonal populations of fibroblasts, one with normal enzyme activity and the other in which enzyme activity is deficient.

TABLE 4. Biochemical abnormalities in AFD

↓	Ceramide trihexosidase	
	Small intestinal mucosa	Brady *et al.* (1967)
	Serum and plasma	Mapes *et al.* (1970)
		Malmqvist *et al.* (1971)
↓	Alpha galactosidase	
	Fibroblasts	Romeo & Migeon (1970)
	Leucocytes	Kint (1970)
		Clarke *et al.* (1971)
		Flynn *et al.* (1972)
		Bacchus (1972)
↑	Ceramide trihexoside	
	Lymph nodes	Brady *et al.* (1967)
	Intestinal mucosa	Brady *et al.* (1967)
	Cultured fibroblasts	Matalon *et al.* (1969)
	Serum	Vance *et al.* (1969)
		Malmqvist *et al.* (1971)
↑	Ceramide trihexoside } Ceramide dihexoside }	
	Kidney	Sweeley & Klionsky (1963)
	Urine	Urbain *et al.* (1967)
		Wolfe *et al.* (1970)
		Desnik *et al.* (1970)
		Clarke *et al.* (1971)
		Malmqvist *et al.* (1971)
		Flynn *et al.* (1972)

Arrows up, increase; arrows down, decrease.

HISTOLOGY

One of the earliest and clinically most significant changes occurs in blood vessels particularly in the smooth muscle of the media of arteries throughout the body. These changes can usually be demonstrated in a deep skin biopsy.

Vacuolation is present (Fig. 1) which on polaroscopic examination is doubly refractile. Lipid granules giving a positive reaction to PAS and Sudan black B may be seen in the vacuoles. Similar granules with or without vacuolation may be present in the endothelium, in pericytes and in smooth muscle throughout the body including the heart. The renal glomeruli and distal renal tubules are also regularly affected. Similar changes may occur in connective tissue cells, ganglion cells and in the interstitial cells of the testis.

On electron microscopy (EM) lamellar bodies are present, the lamellae here being 5·2 nm in width. Denser bodies with the EM characteristics of neutral fat may also occur (Fig. 2a and b). Similar EM appearances have been noted in other reports (Hartley & Miller, 1963; Hartley *et al.*, 1964; Hashimoto, Glass & Lever, 1965; Frost, Tanaka & Spaeth, 1966b; Mullem & Ruiter, 1966, 1968, 1970; Caulet *et al.*, 1967; Sagebiel & Parker, 1968; Perrelet *et al.*, 1969).

The precise nature and derivation of the changes seen on conventional and on electron microscopy have not been fully elucidated. Lehner & Adams (1968) have demonstrated a glycolipid and free cholesterol in the arterial wall and have produced evidence to suggest that the lipid is formed *in situ* rather than by filtration from the plasma. Although ceramide trihexosidase has been demonstrated in lysosomes a primary lysosomal defect as suggested by Hashimoto *et al.* (1965) remains unproven.

In a recent and, to the best of my knowledge, the first report of an endometrial biopsy in AFD, Dr Tighe has demonstrated characteristic changes in stromal capillaries and in epithelial cells. In this patient these changes must have occurred within a maximum time interval of 18 days (Fig. 3a and b).

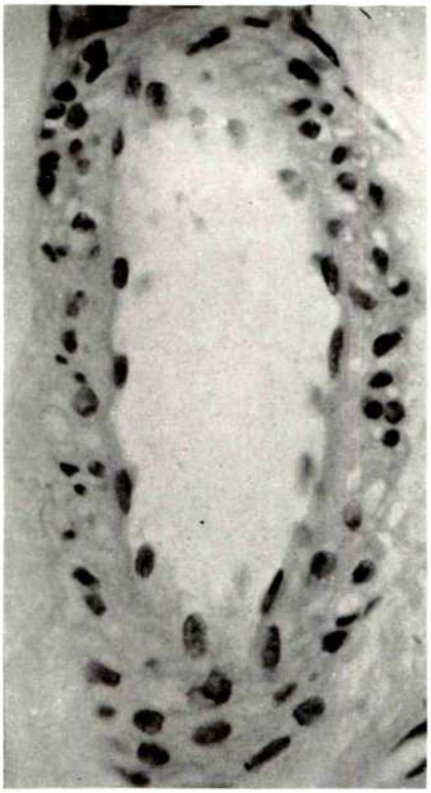


FIGURE 1. Vacuolation in media of skin artery (× 550).

CLINICAL

The clinical features of AFD which I propose to describe today concern primarily twenty-one patients reported by Wise, Jellinek and myself in 1962, (Wise, Wallace & Jellinek, 1962) together with a further thirty-six patients whom I have seen subsequently. Since AFD is imperfectly expressed in females, I propose to deal primarily with manifestations in males. I also propose to restrict detailed observations to manifestations in the skin, kidney and central nervous system.

Skin and mucous membranes

Abnormal skin reactions to vasodilators and vasoconstrictors are common, (Von Gemmigen, Kierland & Opitz, 1965) the cause remaining ill-defined and in particular the significance of damage to blood vessels and to the autonomic nerve supply.

The characteristic eruption consists of superficial blood-filled cavities which may project above the surface as roughly hemispherical papules usually between 0·5 and 2 mm in diameter. Occasionally they may be so small as to be difficult to detect. The colour usually dark red and sometimes almost black, is seldom completely abolished by diascopy. The surface is shiny and macroscopic hyperkeratosis is uncommon except in the largest elements, which may appear almost leaden. Examination with a hand lens shows that many lesions are made up of several vascular foci these being more obvious on microscopy and well described by Anderson as angiectases predominantly in the papillary layer of the dermis. Hyperkeratosis is usually slight. The endothelial cells lining these angiectases are alkaline phosphatase negative suggesting a venous origin. The precise cause of these angiectases and of their distribution remains obscure.

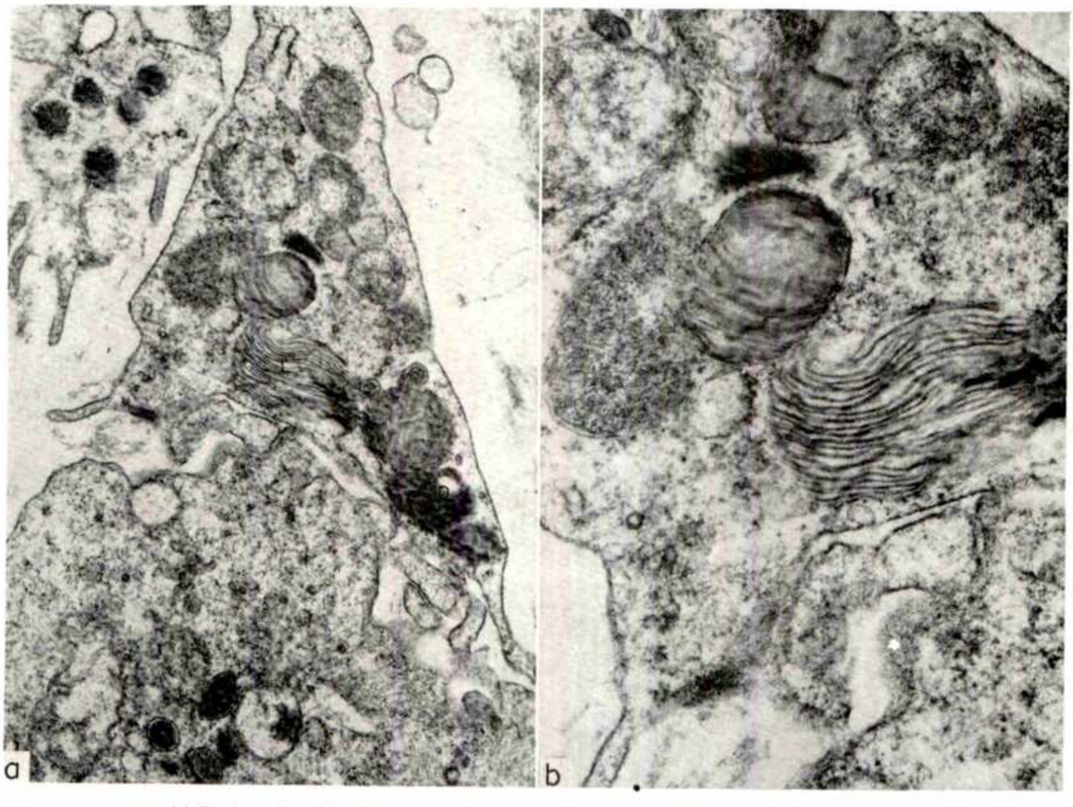


FIGURE 2. (a) Pericyte in skin showing lamellar (L) and denser (D) bodies (× 30,000). (b) Lamellar body as in (a). Lamellae 5·2 nm wide (× 40,000).

The fully developed eruption is most profuse in the area covered by the bathing drawers of a bygone age, particularly over the hips and iliac crests, thighs and buttocks, shaft of the penis, scrotum and the lumbo-sacral area (Fig. 4). The safe seclusion of the umbilicus affords a favourite haven for this eruption. Lesions elsewhere tend to be smaller and may occur on the upper trunk, particularly on the sides of the chest and on the dorsum of the forearm just below the elbow. Occasional lesions may also occur on the upper part of the arms, the dorsum of the fingers and toes and especially around the nail folds. The face, except for a small area below the chin, the scalp, ears and the nail beds, are seldom affected.

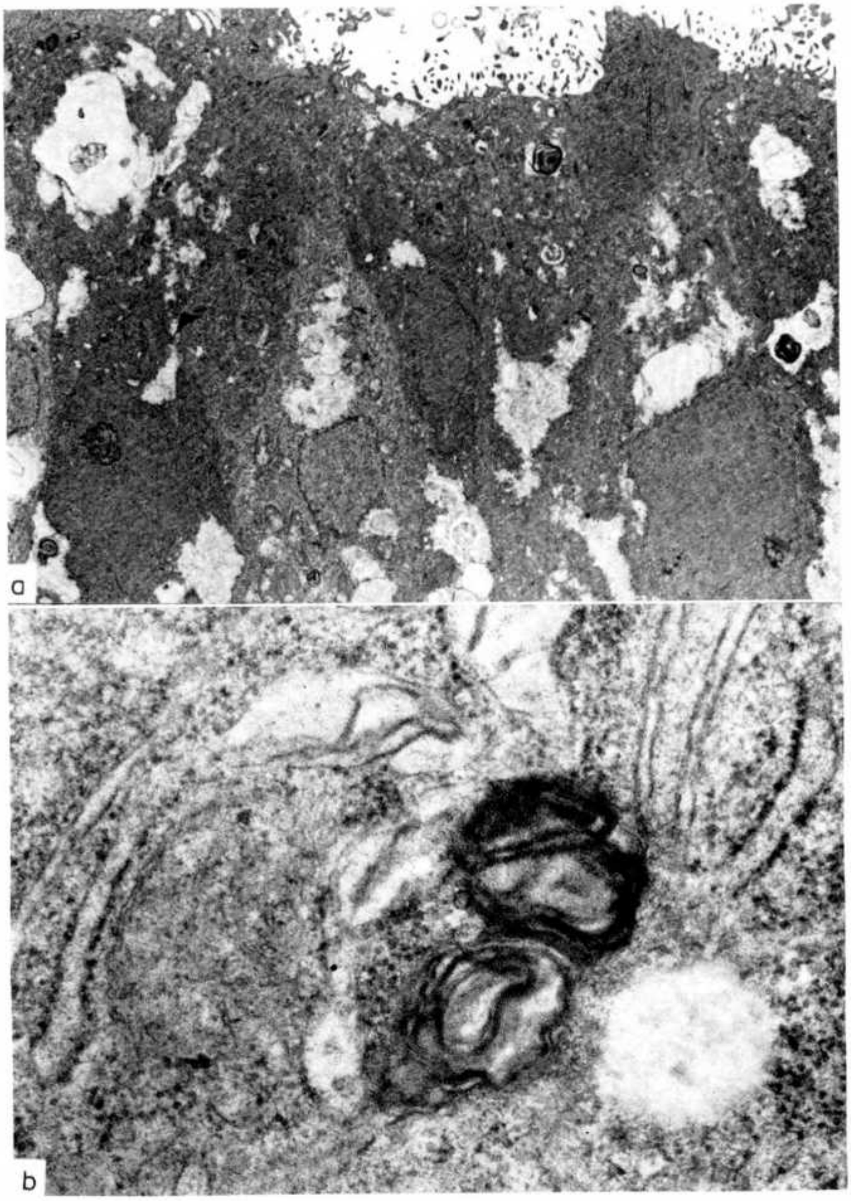


FIGURE 3. (a) Endometrial biopsy (18th day of 28-day cycle). Lamellar bodies in epithelial cells. × 3000. (b) Endometrial biopsy as in (a) showing detail of lamellar body in an epithelial cell (× 54,000).

The eruption frequently occurs in clusters particularly in the bathing drawers area with as many as a hundred in a cluster. In adult males the total number of lesions may run into thousands. In one patient a characteristic eruption appeared on the thigh 2 months after a blow by a cricket ball. A similar eruption has been reported following a hot water bottle burn.

Although retrospective histories often suggest that the eruption began in early childhood, in patients personally examined in this series characteristic lesions were uncommon before puberty, usually some years after the onset of other symptoms. The eruption is seldom conspicuous before the late teens. Similar findings have been reported by Johnston, Weller & Warland (1968), de Groot (1970) and by Flynn *et al.* (1972). Occasionally the onset may be delayed for several years. The eruption tends to become more profuse during the third and fourth decades. Individual elements develop over a period of months to years, shedding usually taking place by thrombosis. Significant bleeding does not occur.

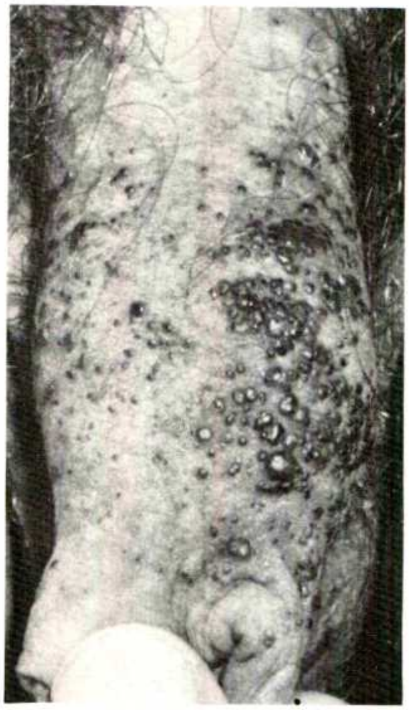


FIGURE 4. Unusually profuse eruption on penis.

The profusion of the eruption bears no relation to the degree of systemic disorder. There are several doubtless authentic reports of adult males with systemic manifestations of AFD but in whom skin lesions were not seen. Dermatologists are notoriously humble and I know therefore that I shall not be thought guilty of arrogance if I disclose that in several patients, my dermatological colleagues and I have been able to demonstrate the characteristic eruption previously overlooked by our friends in more esoteric disciplines. The eruption is present in less than a quarter of adult females with AFD and is usually very slight.

A few lesions may be present on any part of the buccal mucosa, on the fauces, pharynx and gums but seldom on the dorsum of the tongue. The eruption may also occur on the lower lip occasionally as two symmetrical clusters. Angiectases have been recorded in the gastro-intestinal, respiratory and renal tracts at autopsy (Scriba, 1950) but were not seen in any autopsies in this series.

In addition to the characteristic lesions, telangiectasia frequently occurs as a background to the eruption or may occur independently. Corn-like hyperkeratoses have been recorded by Fessas, Wintrobe & Cartwright (1955) and have been seen on the elbows in two patients in this series. Evanescent areas of vasculitis have also been reported (Frost, Spaeth & Tanaka, 1966a) and occurred in one patient in this series.

I do not deem it the function of this lecture to dwell on dermatological minutiae, however significant, and I shall therefore deal but briefly with eruptions that may cause diagnostic confusion. I should like to emphasize that none show the histochemical and electron microscopic changes characteristic of AFD. Firstly, other angiokeratomata: the fairly common Fordyce's angiokeratoma of the scrotum may at first sight mimic AFD but on stretching the skin the lesions are seen to be related to underlying venules. Moreover, there are no lesions on the penis or elsewhere. The peripheral distribution of the angiokeratoma of Mibelli associated with a poor circulation and the pattern of the rare angiokeratoma circumscriptum usually suffice to distinguish them from AFD. The individual lesions of angioma serpiginosum may occasionally mimic AFD. In this disorder which is much commoner in women than in men, the eruption is well developed before puberty and occurs predominantly on the legs. Clustering of lesions does not occur. Purpura with which the eruption of AFD has frequently been confused is or should be readily excluded by the evanescence of individual purpuric lesions. Banal minute angiectases frequently overlooked are not uncommon on the upper part of the trunk and arms in healthy people from late adolescence onwards and may occur in an eruptive form at the menopause. They frequently occur in association with so-called senile angiomata of which they may be a variant. Clustering of lesions does not occur and they seldom cause any difficulty in diagnosis except in women. Senile angiomata, alias Campbell de Morgan spots are usually distinguishable by their larger size and distribution although they often occur in association with AFD. In Osler's hereditary haemorrhagic

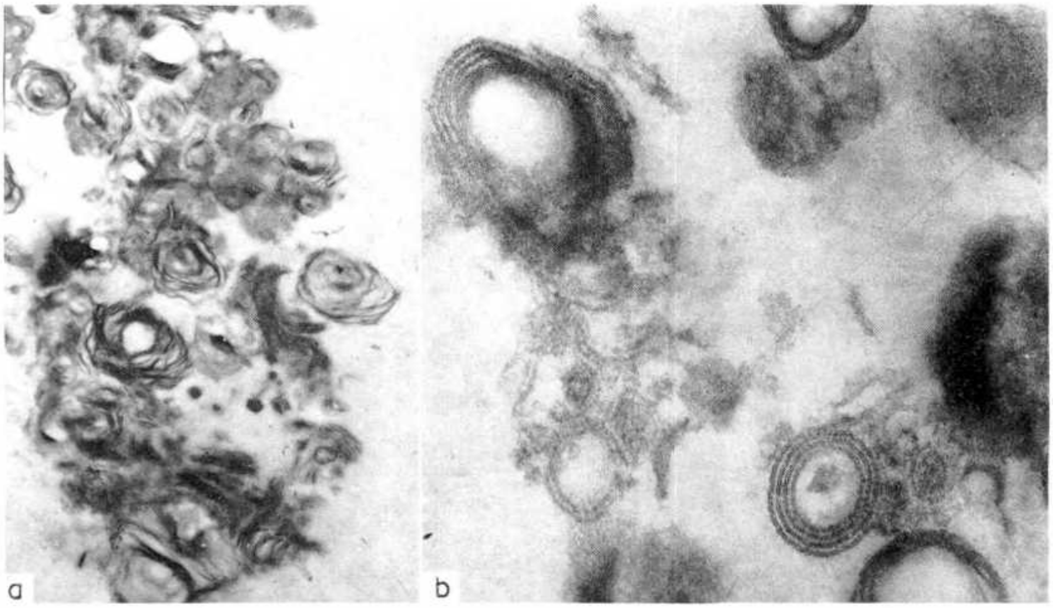


FIGURE 5. (a) Urine sediment. Lamellar bodies of varying density. ♂ aet 17. No proteinuria. (b) Urine sediment as in (a). Lamellar bodies (× 64,000).

TABLE 5. Sites of affected cells in the nervous system (Steward & Hitchcock, 1968)

- (A) Spinal cord
    1. Dorsal root ganglia
    2. Intermediate grey column
- (B) Brain stem
    3. Dorsal efferent nucleus of the vagus
    4. Salivatory nuclei
    5. Nucleus ambiguus
    6. Mesencephalic nucleus of the 5th nerve
    7. Substantia nigra
    8. Reticular formation
- (C) Cerebral hemispheres
    9. Hippocampus (pyramid cells)
    10. Amygdala (basal part of basolateral complex)
    11. Hypothalamus (a) supra-optic nucleus
        (b) paraventricular nucleus
    12. Substriatal grey
    13. External medullary lamina (of the striatum)
- (D) Autonomic ganglia including Auerbach's and Meissner's plexus

Kocen & Thomas (1970) who observed a moderate depletion of myelinated fibres but no defect in conduction.

Neurological symptoms may be grouped into three stages according to age of onset although considerable overlapping may occur.

Childhood and early adolescence are characterized by the onset of pain which I propose to discuss shortly. Mental defect is uncommon and the cause obscure. Headaches, dizziness and transient strokes resulting in aphasia, unconsciousness, hemiplegias and hemianaesthesia may occur in the twenties and early thirties, often with a normal blood pressure. Partial optic atrophy of sudden onset has led on occasion to a diagnosis of disseminated sclerosis. The pathological changes strongly suggest that most of these symptoms are vascular in origin. In the third stage hypertensive strokes are common. Dementia may also occur irrespective of hypertension, the cause remaining obscure.

Pain

Attacks of pain, often of an excruciating and apparently unique character, occur in 90% of males and in less than 10% of females, usually beginning between the ages of 5 and 15. They occur mainly in

TABLE 6. Neurological symptoms in AFD

Common	Uncommon
(I) Peripheral pains	Mental defect
(II) Headache	Neuromuscular disorder in gut and ureter
Dizziness	Epilepsy
Transient strokes (often normotensive)	Optic atrophy, menière, spastic paraplegia, myoclonus
(III) Hypertensive strokes	Dementia

In addition, to the changes which I have described, pyelonephritis and hydronephrosis with megaloureter may also occur.

The nervous system

Neurological symptoms in AFD are conveniently studied by reference to the pathological changes which are most conspicuous in the neurovascular tree. For the autopsy findings in the nervous system in this series I am particularly indebted to Dr W. Steward. Gross changes may be present characteristically in the arteries, often in normotensive subjects, the carotid, vertebral and basilar arteries being particularly affected (Fig. 8). Hypertensive changes are also common.

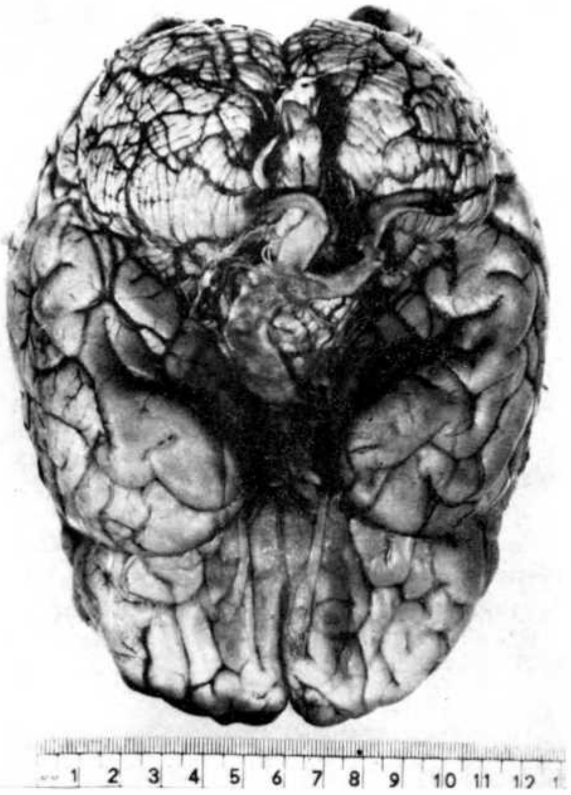


FIGURE 8. Base of brain. Gross dilatation of arteries, especially vertebral, basilar and left carotid. Basilar 12·5 mm wide. ♀ aet 57 (daughter of Anderson's original patient).

Individual nerve cells may also be affected predominantly in the central and peripheral connections of the autonomic nervous system (Rahman & Lindenberg, 1963; Steward & Hitchcock, 1968; Lou & Reske-Nielson, 1971). In the brain and particularly in the cerebral hemispheres, the nerve cells are much less affected than in other sphingolipodystrophies (Jensen, 1966; Lou & Reske-Nielson, 1971).

In addition to these changes Dr Pauline A. Kahn (personal communication, 1971) has also noted axonal degeneration in the posterior root entry zone near the substantia gelatinosa.

Peripheral nerves and nerve sheaths seldom show any abnormality on ordinary microscopy but on electron microscopy changes have been seen in the perineurium. Similar changes have been recorded by Bischoff *et al.* (1968) who also noted degeneration of unmyelinated fibres and by

Progressive vascular degeneration may be followed by wedge-shaped infarcts. Fibrinoid necrosis indistinguishable from the appearances of malignant hypertension may also occur.

On electron microscopy (Fig. 7) there is a widespread accumulation of lamellar and denser bodies especially in the epithelial cells of the glomerulus, the endothelial cells by contrast being less affected or spared. Similar appearances have been noted by Hartley & Miller (1963), McNary & Lowenstein (1965); Rae, Lee & Hopper (1967), Morel-Maroger *et al.* (1966) and Clarke *et al.* (1971).

Impairment of renal function may be detected in early adult life; the ability of the kidney to concentrate the urine may be impaired before there is a reduction in the glomerular filtration rate (Colley *et al.*, 1958). The precise cause of this defect has not been elucidated but it may be relevant that the maximal incidence of AFD at this stage is in those segments of the nephron particularly concerned with urinary concentration. The mechanism of the latter stages and subsequent uraemia is also uncertain. Since extensive vacuolation of the glomeruli and tubules may be present for many years without gross impairment of renal function, vascular changes are probably more significant.

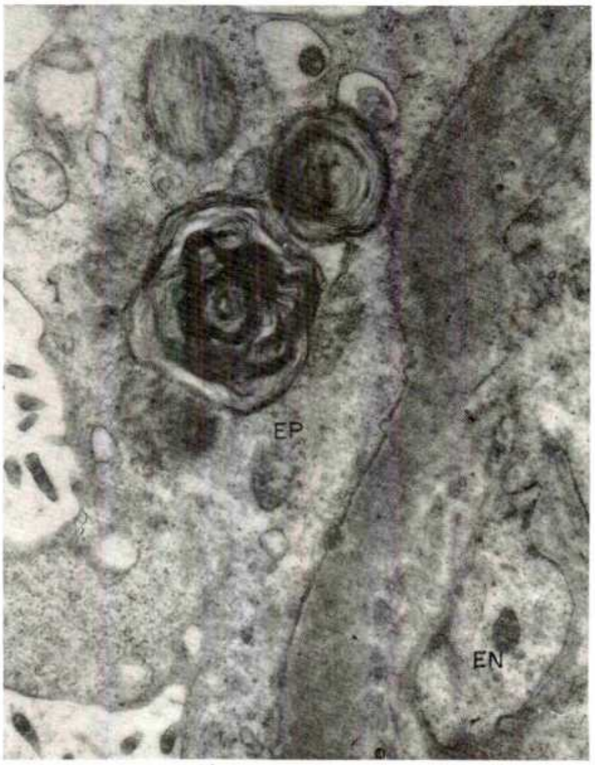


FIGURE 7. Renal glomerulus. Many lamellar bodies in epithelial cell (EP) in contrast to endothelial cell (EN) (× 16,000).

AFD should always be considered in obscure renal failure. In several instances the diagnosis has had to wait appropriate staining of kidneys at autopsy. Familial renal cytodystrophy (Hamburger *et al.*, 1964; Morel-Maroger, Ganter & Verger, 1966) is synonymous with AFD.

Treatment is at present symptomatic. I am indebted to Dr G. Thiel (personal communication, 1971) of Basle for the record of a renal transplant in a patient with AFD which was rejected after 2 years but showed no evidence of AFD.

telangiectasia the lesions are less numerous, larger and more irregular. They are common on the face and dorsum of the tongue, the disorder occurring equally in both sexes.

The urinary tract

Renal failure is the commonest cause of death in AFD. Proteinuria first noticed by Anderson in 1897, may occur in childhood and is almost invariably present in adult males. The amount of protein varies from a trace to more than 4 g/l but seldom causing hypoalbuminaemia. In the early stages the degree of proteinuria may vary greatly from day to day. The urine may contain an excess of hyaline and granular casts and occasionally doubly refractile lipid globules. Macrophages showing honeycomb vacuolation occur less frequently. On electron microscopy of the urinary sediment lamellar bodies may be seen (Duncan, 1970) even before the onset of proteinuria (Fig. 5a and b).

The characteristic histological changes in the kidney (Fig. 6a) were first described in detail by Colley *et al.* (1958) and Wallace (1958) and have since been confirmed by others (Bethune, Landrigan & Chipman, 1961; Henry & Rally, 1963; Wachtel & Mattei, 1964; Funck Brentano *et al.*, 1964; Morel-Maroger *et al.*, 1966; Loeb *et al.*, 1968). Epithelial cells in the glomeruli are finely vacuolated and give a positive reaction to Sudan black B. Occasional cells of Bowmans capsule and many cells in the ascending loop of Henle and in the distal tubules are also affected, the proximal tubules at first being spared. Whilst glomerular vacuolation may be seen occasionally in patients with nephrotic syndrome, in Niemann Pick disease, in some xanthomatoses, and as a complication of heparin treatment (Hall *et al.*, 1971) it is usually limited to a few isolated cells. Extensive vacuolation of the glomeruli and distal tubules appears to be specific to AFD.

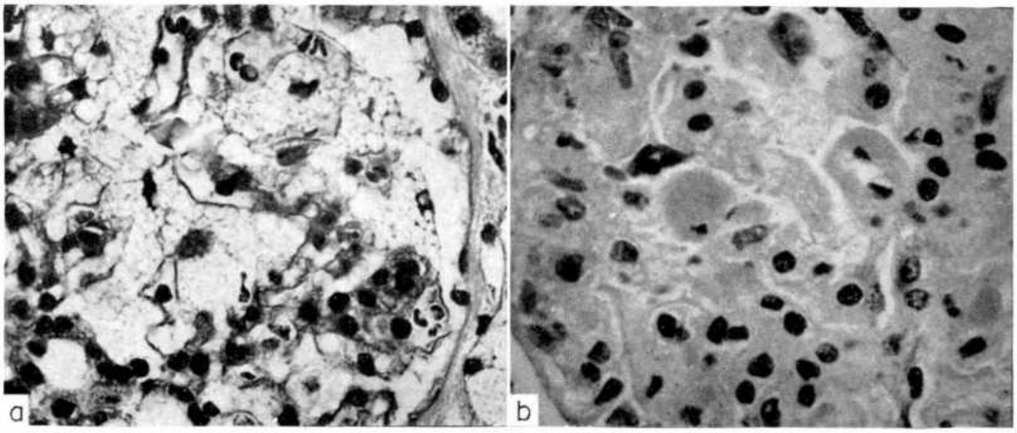


FIGURE 6. (a) Renal glomerulus. Honeycomb appearance due to distension and vacuolation of epithelial cells of tuft. An adjoining distal tubule shows similar appearances. ♂ aet 36. This figure appeared in 1958 *British Medical Journal* **1,** 1266. (b) hyalinization of glomerulus from same patient as in (a). aet 50 (×600).

The blood vessels and particularly interlobular arteries show the characteristic changes of AFD with oedema and degenerative vacuolation of the media and thickening of the intima. Later periglomerular fibrosis is followed by widespread disorganization and shrinkage of the kidney usually accompanied by chronic inflammatory cell infiltration and patchy increase in interstitial tissue. The majority of the glomeruli become hyalinized (Fig. 6b) and are ultimately converted into structureless masses. Even at this stage the presence of lipid can usually be demonstrated. Proximal and distal tubules often show widespread degeneration of their epithelium and dilatation of their lumina.

the fingers and toes and may last from a few minutes to 2 or 3 weeks; attacks may occur at intervals of weeks to months. Sufferers can seldom give a precise description of the pains which appear to have an agonizing burning quality and may be followed by lightning pains or very acute pins and needles. The pain is felt deep in the skin but not in the bones and may persist as a constant niggle. There is little proximal spread although occasionally a burning sensation may affect the whole limb. In an acute phase the sufferer is incapacitated. One intelligent patient said that the best description he could give was that his hands and feet felt full of boiling water. Another likened the pain to a very intense hot ache after exposure to cold. A patient with ophthalmic zoster stated that this pain was mild in comparison with the pains in his fingers and toes. Two patients became drug addicts. The pains are said to have caused one patient to become unconscious. Attempts at suicide have also been reported. Severe pains are at best only mildly relieved by analgesics other than narcotics. In attacks of moderate severity some relief may be obtained by local pressure or moderate cooling. Local vasodilatation may occur during an attack and has led to the nebulous diagnosis of erythromelalgia. Vasoconstriction occurs less frequently. In one patient (Larnder, 1962, personal communication) the pain was preceded by oliguria and oedema. In addition to the pains, ill-defined paraesthesia and cramps are also common.

Prolonged attacks of pain which occur particularly in children, often accompanied by fever, malaise and a raised erythrocyte sedimentation rate, have on several occasions attracted a diagnosis of acute rheumatism and even polyarteritis nodosa. Attacks may occur spontaneously but are most readily provoked and sustained by heat. In a warm environment the patients frequently keep their hands and feet out of the bedclothes or discard socks and shoes. One unhappy little boy reported by de Groot (1970) and originally thought to be a malingerer, declined to go to school during the day in warm weather but was able to play football in the cool of the evening. A long period of chastisement followed until the correct diagnosis was made. Many patients have been diagnosed as neurotic. Less commonly attacks may be caused by cold, the critical temperatures in both heat and cold being specific to each sufferer (de Groot, 1964a). Occasionally pain may occur with intercurrent infection irrespective of fever. Exertion may also cause attacks without any constant relationship to effort, although nature secured a eugenic triumph in one disconsolate swain who complained that he was unable to pursue any sustained courtship. Even slight elevation of the arms as in prayer frequently causes pains in the fingers and sufficed to exempt two patients from a rigid family church parade. When more than one member of a family is affected there is frequently an element of suggestibility in the occurrence and intensity of attacks.

Severe pain in the glans penis was experienced by two patients and was unaffected by erection. Infrequently the tongue may be affected; the pains being provoked particularly by alcohol and hot food. Severe pains in all areas tend to lessen from late adolescence onwards.

The indescribable nature and site of the pain, usually unaccompanied by physical signs appear to set it apart from acroparaesthesia, neuropathies, ischaemic and thalamic syndromes. Causalgia may simulate the pain of AFD but unlike AFD is associated with trophic changes and is sometimes relieved by sympathectomy. Further investigations were carried out in order to try to determine the cause of the pain, the results with few exceptions being in agreement with other reports. Examination of the central nervous system including cutaneous sensation was normal. Serum creatine, phosphokinase and
dolase were normal. Vascular flow studies during and in between attacks were inconclusive. Electro-
cephalography and electromyograms were normal although mild abnormalities have been reported
isser & de Groot (1970). Little or no relief of the pain was obtained by analgesics other than
or by antihistamines, corticosteroids, automatic mimetic agents, vasoconstrictors, vaso-
te ganglion block and bilateral sympathectomy. Significant relief was obtained by
arterial occlusion until the disappearance of cutaneous sensation except for repeated

and heavy pinprick. The effect of pethidine is unlikely to have been caused by its action on smooth muscle since muscle relaxants gave no relief. Centrally acting agents such as chlorpromazine were largely ineffective. Two patients derived relief from tegretol. Recently, Lockman, Krivit & Desnick (1971) have reported relief of pain with diphenylhydantoin and I have been able to confirm some relief in two out of four patients. The mechanism of the analgesic effect of these drugs is not known.

The cause of the pain in AFD remains obscure. The effect of arterial blockage suggests that the origin of the pain is in part at least at the periphery. Many questions remain unanswered. What is the explanation for the quality of the pain? How do heat, cold and exertion cause an attack? What is the cause of the fever that frequently accompanied acute pain? Why do pains tend to lessen with age? What is the significance of the pathological changes in the nervous system?

Neurophysiological problems of pain are still far from resolved and well beyond the scope of this lecture. I suggest that pain in AFD with its characteristic clinical and pathological features affords an excellent and perhaps unique model for investigation.

Eyes

The conjunctival vessels may be dilated and tortuous particularly in the area covered by the lower lid. The most characteristic change in the eye is a symptomless superficial corneal dystrophy often revealed only by careful slit lamp examination, preferably with a reflected light on a dark background. The typical appearances first described by Weicksel (1925) are faint grey, occasionally mustard coloured wedges extending inwards from the limbus and narrowing to a point usually just below the centre of the cornea (Fig. 9). They have curved outlines and are often associated with wavy streaks or small blotches. Occasionally there is a faint uniform cloud over much of the cornea. The importance of these changes in the diagnosis of AFD was first stressed by Wise *et al.* (1962), since they are mimicked only b chloroquin keratopathy. In this series they were present in two boys aged 6 but have been reporte a boy aged 6 months (Spaeth & Frost, 1965) and in a girl aged 3 (Colombi *et al.*, 1967). With exceptions they were present in all patients, male and female, over the age of 18 in thi again with few exceptions have been noted in all recent reports of AFD in adults (Rah berg, 1963; Grace, 1966; Velzeboer & de Groot, 1971). The pathogenesis of this d

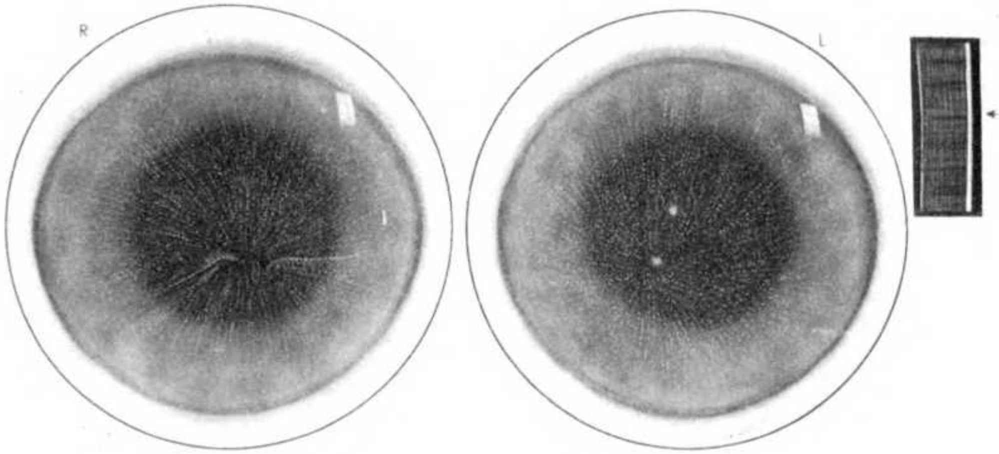


FIGURE 9. Corneas of a man aged 26 showing the characteristic slightly curved linear opacities.

obscure. Hyperplasia of the central epithelial cells of the cornea associated with well demarcated areas of oedema have been noted, Bowman's membrane and the substantia propria appearing normal (Wallace, 1958).

In an EM study of the eye in one patient in this series and to be published later, Professor Norman Ashton has demonstrated lamellar bodies in the corneal epithelium, together with typical deposits in the retinal vessels. Weingeist & Blodi (1971) have demonstrated similar changes in the corneal epithelium with reduplication of the basement membrane and amorphous material between the basement and Bowman's membrane.

Dilatation and tortuosity of retinal vessels, especially veins, is common and may be present in normotensive subjects, even in childhood (Fig. 10). Irregularity of calibre may also occur giving a beaded appearance and is well shown in a fundus photograph after intravenous injection of a fluorescent dye, the latter being retained within the vessel walls and showing no impairment of the blood retinal barrier. Later hypertensive changes may be superimposed. The lens opacities reported by Von Gemmigen *et al.* (1965) and by Spaeth & Frost (1965) have not been observed in this series.

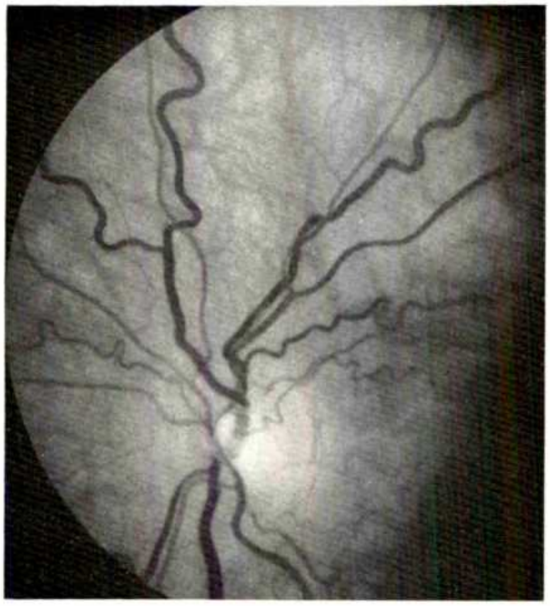


FIGURE 10. Retina. Dilatation and gross tortuosity of veins. ♂ aet 14.

Oedema

Oedema, initially transient, is common, particularly in the summer and may be the presenting symptom. The ankles are usually affected and less frequently the hands and face. The oedema frequently precedes any significant renal, cardiac or local cause and suggests an abnormal permeability of the capillaries in the skin and subcutaneous tissue.

nen

by *[illegible]hidrosis*

narcotic[illegible]sis is also common and may cause much distress in warm weather or with exercise. Obser-
dilators, stella[illegible] a functional impairment in sweat glands (de Groot, 1964a; Von Gemmigen *et al.*, 1965)
pethidine and by[illegible]ect in their number.

Fever

Fever lasting for days to weeks may accompany attacks of pain or may occur independently. It does not respond to antibiotics or aspirin and only rarely to cortisone. The cause remains obscure.

Gut

Episodes of acute abdominal pain with or without diarrhoea have been reported on several occasions and occurred in four patients in this series. In three of these four patients segmental colitis was present and in one accompanied by narrowing of the ilio-colic junction. The cause of these symptoms is uncertain and in particular the significance of impairment of the autonomic nerve supply. Lipid deposits may also occur in the blood vessels of the gut wall (Fig. 11).

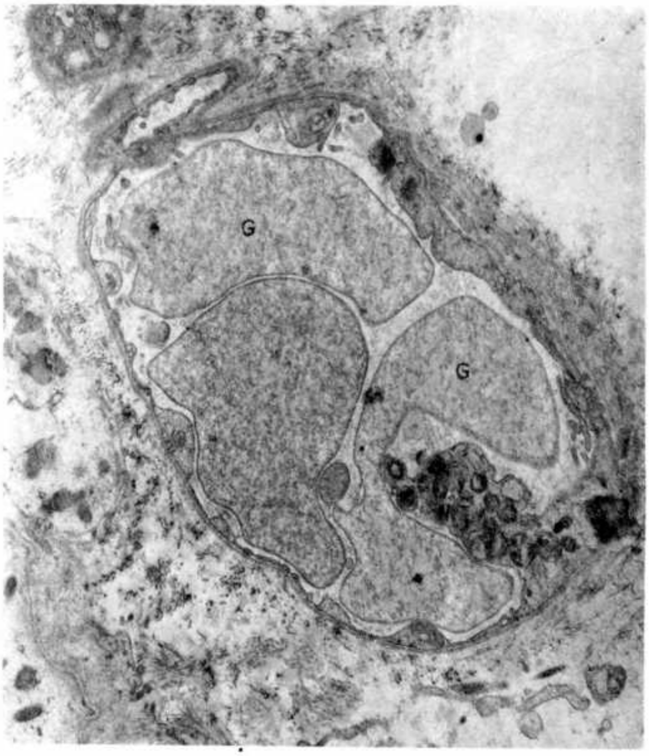


FIGURE 11. Jejunal wall. Lipid deposits in endothelial cell of capillary with ghost (G) red cells in lumen (× 6500).

Heart

The characteristic changes of AFD have been demonstrated in all autopsies in the coronary arteries and in the myocardium (Ferrans, Hibbs & Burda, 1969). Clinical manifestations may occur in the second and third decades. Hypertension and left ventricular hypertrophy are common although seldom severe except in the later stages of renal failure. Abnormal electrocardiograms, angina and coronary thrombosis may also occur, frequently in normotensive subjects. Rarely there may be mitral and aortic valve disease.

Blood

Moderate anaemia, usually hypochromic, is common, the cause often remaining obscure. Dr Tighe and Dr Creamer have noted occasional 'ghost' red cells (Fig. 11) on electron microscopy. Since globoside is an important constituent of the red cell envelope and its degradation impaired in AFD, at my suggestion Dr A. J. Grimes has kindly investigated the red cells in three patients without detecting any abnormality. Reticulocytosis is uncommon and $_{51}$Cr studies in two patients in this series were normal. A raised erythrocyte sedimentation rate of obscure origin is also common particularly in association with pain or fever. Hypogammaglobulinaemia has been reported in three members of a family by François, Snacken & Stockmans (1968) and in another patient by Urbain, Philippart & Peremans (1969).

Lungs

The changes of AFD have been noted in the lung (Bagdade *et al.*, 1968), with impairment of diffusing capacity. There is an enhanced incidence of asthma, chronic bronchitis, emphysema and spontaneous pneumothorax.

General and physique

Most adult males with AFD seldom feel well and general physique is often poor. In some families thick lips and coarse features are common and may be associated with a long gangling stance and rarely with macroglossia. Sexual maturation may be retarded and beard growth indifferent. A painless arthropathy of the terminal interphalangeal joints associated with degenerative change is fairly common. Impaired mobility may occur in other joints but severe joint pain is infrequent. Occasional abnormalities include parrot-beaking of finger nails, avascular necrosis of the head of the femur (Pittelkow, Kierland & Montgomery, 1955; Wise, 1972, personal communication). Polymyositis followed by fibrosis has also been reported (Wise *et al.*, 1962), and one instance of myopathy (Denk & Sollberg, 1966).

HEREDITY

Colley *et al.* (1958) reported the first instance of AFD in a female who was subsequently shown by Wise (1960) to be the daughter of Anderson's original patient. Wise *et al.* (1962) reviewed the genetics of AFD in eight families and conluded that the gene was likely to be sex linked, with occasional penetrance in the heterozygous female and complete penetrance in the hemizygous male. This conclusion has since received support in other studies (Opitz *et al.*, 1965; Johnston *et al.*, 1968). No evidence of chromosomal defect has been found (Jensen, 1966; Caulet *et al.*, 1967; Eberle & Denden, 1969); the evidence also suggests that the locus for AFD is not far from those for the Xga blood group and for the deutan type of colour blindness (Johnston *et al.*, 1969).

DIAGNOSIS

There are now reports of over 200 patients with AFD the majority having been recorded in the last 10 years. It may be that only a minority of patients with AFD are ever correctly diagnosed.

In this series, and omitting the patients in whom the diagnosis had to await autopsy findings, the average duration from the onset of symptoms to correct diagnosis has been 18 years, the eruption usually having been overlooked, misdiagnosed or disregarded. I hope therefore that I may be forgiven for a brief recapitulation of the more important diagnostic aids. I suggest that AFD should always be considered whenever the cause remains obscure in pain in the extremities, in nervous disorders or in chronic renal failure. AFD also merits the attention in the diagnosis of obscure fever and heat intolerance.

In males the eruption is usually present by adult life but is often absent or very slight in childhood and adolescence. In women the eruption is present in less than a quarter and seldom conspicuous.

When symptoms are present in males or females the characteristic corneal dystrophy and lipid deposits in a skin biopsy are almost invariable from childhood onwards and afford the simplest method of diagnosis. In order to demonstrate the lipid deposits I wish to stress the importance of the correct technique (Ruiter, 1954, 1957) namely that after calcium formalin fixation the tissue remains in 3% potassium dichromate solution for a week, the dichromate solution being renewed every third day. In the detection of AFD in boys before the onset of symptoms or of symptomless carriers before adult life the urinary excretion of ceramide dihexoside and ceramide trihexoside may give the earliest clue. The finding of lipid skin deposits in a girl of 5 and a boy of 6 (de Groot, 1970) suggests that the typical EM appearances may also prove to be present in early childhood. In adult carriers, even if symptomless, corneal dystrophy is usually present.

TABLE 7. Diagnostic criteria in AFD

Eruption	Ceramide trihexoside/dihexoside in urine
Corneal dystrophy	Urine sediment (lipids, EM)
Skin biopsy (lipids, EM)	Alphagalactosidase in leucocytes and fibroblasts

PROGNOSIS AND TREATMENT

The clinical picture and prognosis vary greatly although less within afflicted members of a family. The average expectation of life in males is 42 years. I am indebted to Dr A.W.Johnston (personal communication, 1972) for a report of the oldest man with AFD that I have been able to discover, a patient in his care who died just short of his 65th birthday. In less than 10% of women with AFD is their expectation of life diminished.

Treatment hitherto has achieved little beyond slight relief of symptoms. The recent discovery of a deficiency of ceramide trihexosidase suggests a more helpful future although the many problems that have to be overcome in the production of the enzyme and the possible difficulties of replacement treatment are a warning against undue optimism. Nevertheless, the recent work of Mapes *et al.* (1970) is encouraging. They infused two AFD patients with normal plasma to provide active enzyme. Enzyme activity proved to be greater *in vivo* than *in vitro* and its effect was still detectable at the end of a week.

Most patients with AFD readily agree to any reasonable investigation to help future sufferers rather than in the expectation of personal relief. They show courage which cannot fail to command respect and richly merit a more hopeful future.

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I am particularly indebted to Professor M.Ruiter for his help and outstanding contributions in the understanding of Anderson-Fabry disease, to Dr D.Wise for his never failing stimulus and for his work on the genetics of this disorder, to Professor F.T.G.Prunty for his help with the chemistry of the dyslipidoses, to Dr J.Tighe, to Professor M.S.R.Hutt and Mr A.Clark for the histological studies and to Dr W.Steward for his detailed description of the nervous system in autopsy material.

I am also indebted to so many colleagues who have allowed me to examine many of the patients described in this paper or who have helped me in other ways, that any selection of names is invidious. I should, however, like to acknowledge particularly the help that I have received from Dr R.J.Cairns, Dr M.P.Crick, Dr P.C.Farrant, Dr P.Frost, Dr W.P.de Groot, Dr P.Hall Smith, Dr M.L.Johnson, Dr N.F.Jones, Dr C.A.Keele, Dr P.Rhodes, Dr T.W.E.Robinson, Dr M.D.Sanders, Dr M.E.Smith, Dr I.W.Whimster and Dr J.Woodall. My warmest thanks are also due to Mr T.W.Brandon and Mr R.Phillips and the photographic departments of St Thomas's Hospital and St John's Hospital for Diseases of the Skin.

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British Journal of Dermatology (1973) **88**, 25.

Biphasic amyloidosis: link between macular and lichenoid forms*

MARTIN H. BROWNSTEIN, KEN HASHIMOTO† AND
GERALD GREENWALD

Departments of Pathology and Dermatology, New York Medical College–Metropolitan Hospital Center, New York, N.Y. and †Memphis Veterans Administration Hospital and the Division of Dermatology, Department of Medicine, Memphis, Tennessee

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SUMMARY

A woman manifested lichen amyloidosus of the right lower limb and macular amyloidosis of her left leg with congenitally impaired cutaneous sensation. Another patient had bilateral lichenoid amyloidosis of her legs. Unilateral triamcinolone injections produced the picture of macular amyloidosis in the treated leg. Light and electron microscopic studies of biopsies from both patients showed similar amyloid deposits in the papillae; the significant difference was epidermal hyperplasia in the lichenoid lesions. We suggest the term biophasic for the existence of both macular and lichenoid amyloidosis in the same patient.

The dramatic clinical picture of intensely pruritic keratotic papules in lichenoid amyloidosis contrasts sharply with the subtle pigmentary changes found in the macular variety. Nevertheless, it has recently been suggested (Brownstein & Hashimoto, 1972) that these conditions may represent the same basic disease process, and that the essential differences are due mainly to secondary hyperplastic changes of the epidermis.

Our purpose here is to present evidence of an intimate relationship between these disorders by describing two patients with biphasic amyloidosis—lichen amyloidosus of one lower limb and macular amyloidosis of the other.

CASE REPORTS

Case 1

A Puerto Rican woman aged 47 complained of a pruritic eruption that had been present on the right lower limb for several years. She was born with orthopaedic deformities and neurological defects of the left leg, and had never been able to walk normally.

On examination there were several plaques between the right ankle and mid-tibial area, consisting

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Requests for reprints: Dr Brownstein, Post Office Box 571, Great Neck, New York, 11022, U.S.A.

of closely aggregated, keratotic, dome-shaped and acuminate, skin-coloured to brown papules of about 3–5 mm in diameter. The left lower limb was shorter than the right, the muscles of the calf were atrophic, and there was severe talipes arcuatus deformity. The skin was flat and smooth to the touch and manifested barely discernible speckles of brown macular pigmentation. Pain and thermal sensation were impaired below the level of the left mid-thigh; touch, vibration, and position sense were normal. The Achilles tendon reflexes were 1+bilaterally; the knee jerk on the right was normal, but that on the left could not be tested because of local changes in the soft tissues.

The clinical diagnosis was lichen amyloidosus of the right, and macular amyloidosis of the left lower limb. A biopsy specimen from the right pretibial area disclosed epidermal hyperplasia and eosinophilic masses replacing the normal connective tissue in the papillary corium. Crystal violet staining (Plate 1) revealed the reddish metachromasia of amyloid. A biopsy specimen from the left pretibial area was studied by light and electron microscopy. Haematoxylin–eosin stained sections showed an essentially normal epidermis with light pink amorphous masses in the dermal papillae. Crystal violet staining (Plate 4) confirmed the clinical suspicion of macular amyloidosis. Examination with the electron microscope showed islands of amyloid in the papillary dermis. On high magnification, typical filaments measuring approximately 8–10 nm were seen.

Case 2

A Puerto Rican woman aged 36 complained of a pruritic eruption that had been present on both shins for several years. Overlying the pretibial regions were dark brown plaques composed of discrete keratotic papules ranging in diameter from about 2 to 4 mm. The left leg (Plate 2) was more severely affected than the right. The results of a general physical examination and routine laboratory studies, including the levels of serum albumin, alpha-1, alpha-2, beta, and gamma globulin were within the normal range. Radial immunodiffusion analysis of the serum disclosed an IgG level of 500 mg% (normal 900–1500 mg/100 ml), IgA of 400 mg/100 ml (normal 160–410 mg/100 ml), and IgM of 180 mg/100 ml (normal 60–100 mg/100 ml).

A biopsy specimen from each pretibial area was bisected and examined with light and electron microscopes. Haematoxylin–eosin preparations showed foci of hyperkeratosis and hypergranulosis with deposition of amorphous material in the subjacent dermal papillae. Crystal violet (Plate 5) and alkaline Congo red preparations confirmed the amyloid nature of the deposits. Examination with the electron microscope showed islands in the upper dermis composed of 6–10 nm stiff, straight amyloid filaments.

During the following 6 months, the left leg only was treated with sublesional injections of triamcinolone acetonide suspension, in a concentration of 5 mg/ml. Five milliliters were given at biweekly intervals. Topical or systemic therapy was not employed. The right leg showed no change. The eruption on the left pretibial region gradually flattened, and pruritus subsided, but flecks of brown pigmentation persisted, and the area assumed the clinical picture of macular amyloidosis (Plate 3). A second biopsy specimen from the left pretibial region was examined with the light and electron microscopes. Sections stained with haematoxylin–eosin showed an essentially normal epidermis with deposits of homogeneous, eosinophilic material in dermal papillae. With crystal violet (Plate 6) the deposits displayed reddish metachromasia. Electron microscopic studies (Fig. 1) showed amyloid islands in the upper dermis. There were no significant alterations in the size and distribution of amyloid deposits, and no change in the morphology of amyloid filaments, as compared to the pretreatment biopsy.

DISCUSSION

The cutaneous amyloidoses may be conveniently divided into primary and secondary varieties of

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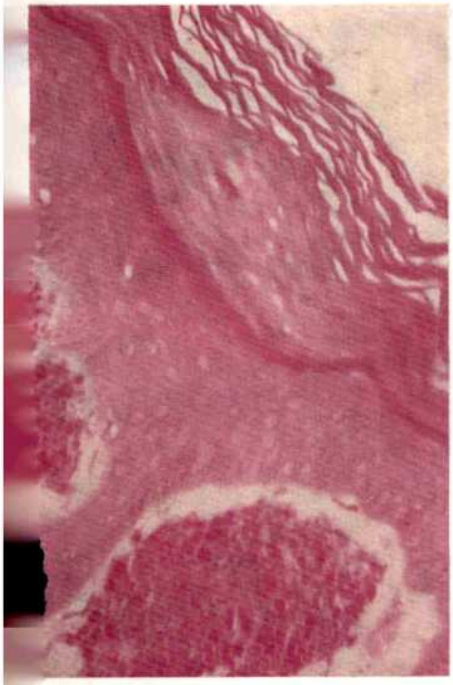

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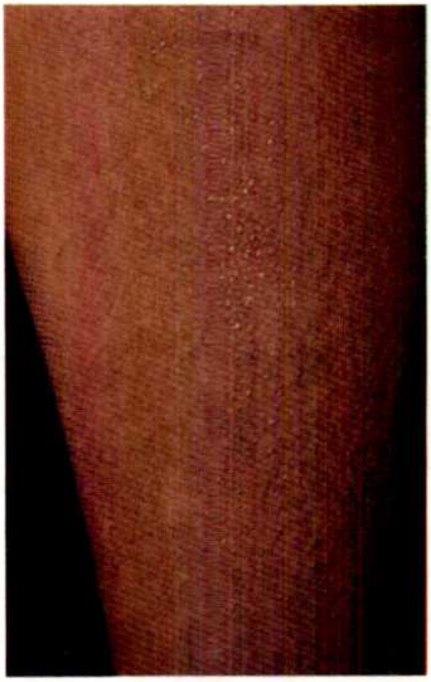

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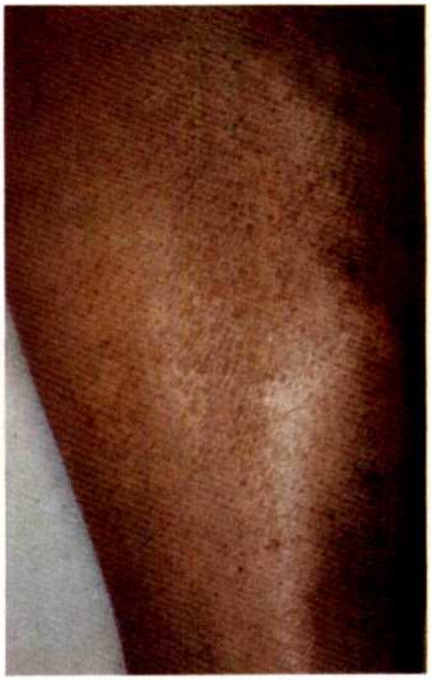

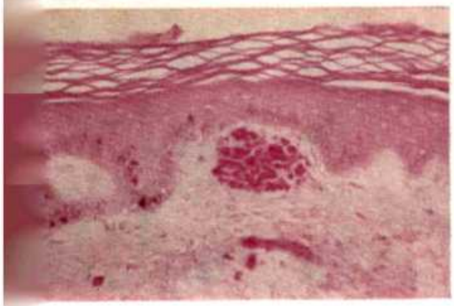

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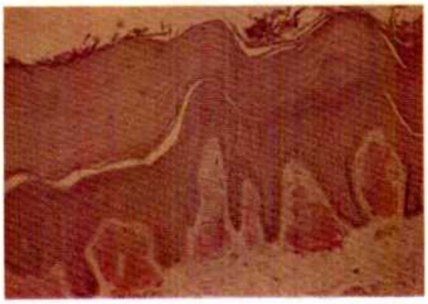

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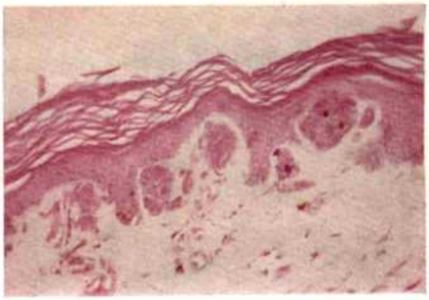

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PLATE 1. Case 1. Lichen amyloidosus of right pretibial area; epidermal hyperplasia and metachromatic material in papillary corium. Crystal violet (× 750).

PLATE 2. Case 2. Lichen amyloidosus of left shin before therapy.

PLATE 3. Case 2. Macular amyloidosis of left shin after therapy.

PLATE 4. Case 1. Macular amyloidosis of left pretibial area; normal epidermis and amyloid deposits in papillary dermis. Crystal violet (× 500).

PLATE 5. Case 2. Lichen amyloidosus of left shin before therapy. Epidermal hyperplasia and amyloid deposits in dermal papillae. Crystal violet (× 500).

PLATE 6. Case 2. Macular amyloidosis of left shin after therapy. Normal epidermis and amyloid deposition in papillary bodies. Crystal violet (× 500).

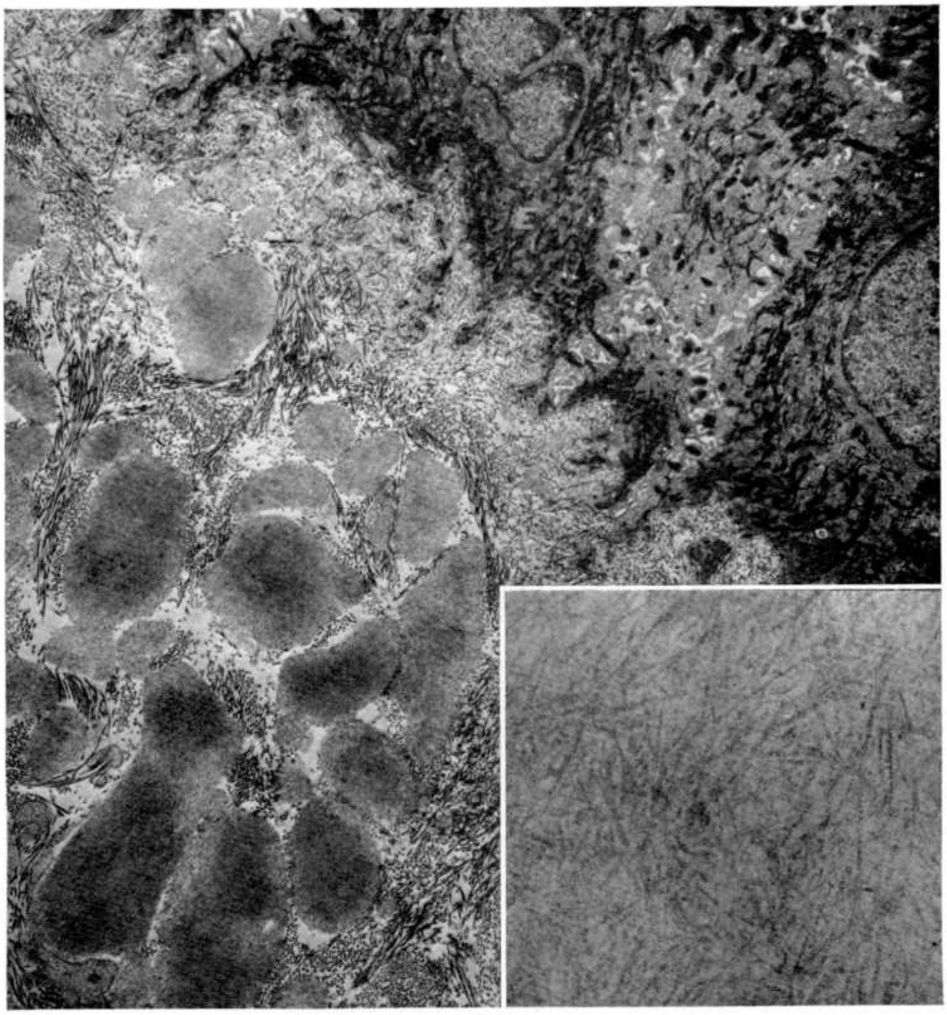

FIGURE 1. Amyloid islands persist in the usual subepidermal location following corticosteroid injections and normalization of hyperplastic epidermis. E, epidermis. Case 2 (×2,275; insert ×58,450).

systemic and localized disease (Brownstein & Helwig, 1970a, 1970b). Primary localized cutaneous amyloidosis is not meaningfully related to any other disorder, and is not associated with the deposition of amyloid in other organs. It has been subdivided into tumefactive, lichenoid, and macular varieties. In the tumefactive type, the epidermis is usually thinned, and the dermal papillae are not prominently affected. Large dermal and subcutaneous deposits of amyloid usually invade the walls of blood vessels.

In lichen amyloidosus there are usually symmetrically distributed intensely pruritic papules on the lower limbs. The essential changes with the light microscope are epidermal hyperplasia and amyloid deposits that are limited to the papillary corium. With the electron microscope (Shapiro, Kurban & Azar, 1970; Hashimoto & Yoong Onn, 1971), amyloid appears in close relation to fibroblasts in the papillary dermis; amyloid fibrils have also been observed within the cytoplasm of fibroblasts and in the intercellular spaces of the epidermis.

Macular amyloidosis (Black & Wilson-Jones, 1971; Brownstein & Hashimoto, 1972) shows a predilection for the thighs, arms, and back. The eruption consists usually of mildly pruritic, symmetrically distributed, brown, rippled macules. On histopathological examination, the epidermis is essentially normal, but amyloid deposits in the papillary corium are similar to those found in lichen amyloidosis. Ultrastructurally, islands of amyloid, many of them related to fibroblasts, are present in the papillary dermis and extend to the dermoepidermal junction. The identical changes found in the cutis on examination with both the light and electron microscope, have previously suggested that lichenoid and macular amyloidosis represent the same basic disease process (Brownstein & Hashimoto, 1972).

In the present study, two patients with lichenoid and macular (biphasic) amyloidosis were investigated clinically, histopathologically, and with the electron microscope. In the first, lichen amyloidosis appeared in the otherwise normal leg, while macular amyloidosis was found in the congenitally deformed leg with impaired sensation. The second patient manifested bilateral lichen amyloidosis; therapeutic intervention transformed the eruption in one leg to macular amyloidosis.

The demonstration of spontaneous biphasic amyloidosis in one patient and its induction in another has important therapeutic, pathogenetic, and nosologic implications. Active therapy can alleviate pruritus, convert a hyperplastic epidermis to one that is essentially normal, and transform the conspicuous papules of lichen amyloidosis to subtle pigmented macules. While therapy is worthwhile and indicated, it is now possible to define its goals and limitations. Amyloid deposits in the papillary corium are not reversed; treatment must probably be continued in order to minimize pruritus and to prevent the recurrence of lichenoid lesions.

In the patient with spontaneous biphasic amyloidosis, the amyloid deposits in the dermal papillae were not significantly different in biopsy specimens from each leg. The main difference was the presence of hyperkeratosis and acanthosis in the lesions of the leg with normal sensation. The unique spontaneous occurrence of macular amyloidosis in a limb with impaired sensation and lichenoid amyloidosis in that which had previously been normal would appear to be more than fortuitous. Since histologic studies demonstrated similar deposits in each leg, it is likely that the impaired sensation in one limb prevented the development of secondary epidermal changes of lichen amyloidosus.

Multiple factors account for the development of macular amyloidosis in some circumstances and lichenoid amyloidosis in others. The mere presence or absence of pruritus is not the only variable, since moderate pruritus is not infrequently noted in patients with macular amyloidosis. The intensity of pruritus, as well as the anatomic site of amyloid deposits and individual differences of cutaneous reactivity may be important factors. It is well known, for example, that lichen planus and neurodermatitis, as well as lichen amyloidosis, have a tendency to manifest outstandingly hypertrophic forms when they occur on the lower limbs. Indeed, macular amyloidosis in its purest form usually occurs on the back or thighs; lesions on the legs that are macular in their clinical appearance nevertheless show a tendency for modest hyperkeratosis.

We have recently emphasized (Brownstein & Hashimoto, 1972) that the important difference between lichenoid and macular amyloidosis is not the amount of amyloid deposited, but the presence of epidermal hyperplasia in the former condition and its absence in the latter. The patients with bi phasic amyloidosis described in this report provide further evidence of a link between lichenoid and macular varieties.

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Diffuse hair loss and oral contraceptives

W.A.D.GRIFFITHS

Department of Dermatology, St Thomas's Hospital, S.E.1

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SUMMARY

Diffuse loss of scalp hair occurs in some women when they stop taking mixed oestrogen/progestogen oral contraceptives. Nine women had diffuse alopecia while still continuing to take the pill. The relationship between the hair thinning and oral contraceptives is discussed.

Do oral contraceptives cause diffuse loss of scalp hair in women? The association has been reported (Cormia, 1967; Greenwald, 1970; Dugois *et al.*, 1971), and discussed (*British Medical Journal*, 1968; Jelinek, 1970). This study examines the relationship further.

METHOD

The series comprises thirty-one women seen consecutively for diffuse thinning of scalp hair between March 1970 and March 1971. Post-menopausal or pre-pubertal patients have been excluded. All patients were examined personally.

Details of the distribution of the hair loss, the length, colour, calibre and lustre of the hair, state of the body hair, and changes in the nails were recorded.

The investigation of all patients included: haemoglobin and film; serum iron and total iron-binding capacity; Wassermann reaction; protein-bound iodine; tri-iodothyronine resin uptake and total thyroxine, if a patient was taking oral contraceptives.

A biopsy of the scalp was examined in six patients.

To find the prevalence of the use of oral contraceptives, women attending the dermatology clinics whose symptoms did not include hair loss were asked whether they had taken an oral contraceptive in the previous 12 months.

RESULTS

At the time of the study 30% of the 203 women between the menarche and the menopause who attended the dermatology clinic were taking an oral contraceptive, or had taken one in the previous 12 months.

The relationship between diffuse alopecia and taking oral contraceptives is shown numerically in Table 1. There is no statistically significant correlation between the two factors.

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The mean age at the onset of the hair thinning was 26·8 years with limits of 15 and 50, and a median age of 27 years.

The mean age at presentation to the dermatology clinic was 29·9 years, with limits of 16 and 62, and a median age of 29 years. Most women therefore tended to have thinning hair for 2–3 years before seeking advice. 48·5% of the patients had a sibling or parent with thin scalp hair. In three-quarters of those with a positive family history, the relative's hair loss was evident before the age of 30.

The thinning was distributed over the vertex and frontal region, with sparing of the parieto-occipital areas. In the few patients in whom the loss was ascribed to a systemic disorder, the parieto-occipital areas were also involved.

TABLE 1. Number of patients taking oral contraceptives with and without thinning hair

	Not on pill	On pill	Total
Controls	123	49	172
Diffuse alopecia	20	11	31
Total	143	60	203

$\chi^2 = 0{\cdot}624$; $P<0{\cdot}3$.

TABLE 2. Cause of hair loss in nineteen patients not taking oral contraceptives

Post-partum hair loss	5
Malnutrition	1
Amphetamines	1
Diffuse alopecia areata	1
Telogen effluvium (diabetic ketosis)	1
Traumatic?	1
No cause found	9

The proportion of patients with light hair and dark hair was similar in the two groups ($P>0{\cdot}1$). The data on the clinical findings is similar to that recorded in previous investigations (Eckert *et al.*, 1967; Alexander, 1965; Smith & Wells, 1964).

Oestrogen-containing oral contraceptives cause a rise in protein-bound iodine and a fall in the resin uptake of tri-iodothyronine (Winikoff & Taylor, 1966). Thyroid dysfunction is more accurately indicated by calculating a 'free thyroxine index' (Clark & Brown, 1970). No patients had clinical or biochemical evidence of thyroid dysfunction.

Iron deficiency may cause diffuse hair loss, but mixed oestrogen/progestogen contraceptives cause a *rise* in both serum iron and total iron-binding capacity (Burton, 1967; Jacobi, Powell & Gaffney, 1969), and only one patient was found to be iron deficient.

Nineteen of the thirty-one patients with diffuse alopecia had neither taken oral contraceptives nor noticed hair loss related to pregnancy. A cause for the diffuse alopecia was found in ten as shown in Table 2.

Details of the eleven patients who were still taking, or had recently stopped taking oral contraceptives when the hair thinning started are shown in Table 3.

TABLE 3

Case no.	Taking pill when hair loss started	Brand of contraceptive	Pr.	Oe	Duration of medication	Comments
1	No	Gynovlar 21	3	50	3 months	Stopped taking pill 4 months before loss
2	No	Lyndiol	5	150	18 months	Stopped pill 5 months before hair loss
3	Yes	Orthonovin 1/80 Orthonovin 1/50	1 1	80 50	3 months 9 months	Changed pill 5 months before hair loss
4	Yes	Norinyl 1 Demulen 50	1 0·5	50 50	5 months 7 months	Changed pill 3 months before hair loss
5	Yes	Orthonovin S.Q. Minovlar	0 2 1	100 100 50	30 months 7 months	Changed pill 1 month before loss. Also lost hair after two out of three pregnancies
6	Yes	Minovlar Gynovlar	1 3	50 50	8 months 2 months	Changed pill 1 month before loss. Also iron deficiency
7	Yes	Conovid Lyndiol	5 5	75 150	'years'	Missed medication for 1 month, 4 months before hair loss
8	Yes	Sequens	0·5 0·2	80 80	6 months	Took pill for 6 months after a pregnancy. Noticed hair loss about the time she stopped
9	Yes	Ovulen Norinyl 1 Ovulen 50	1 1 1	100 50 50	24 months 12 months 6 weeks	Loss noticed after 12 months on pill
10	Yes	Tova	0 2·5	100	24 months	Loss noticed after 6 months on pill
11	Yes	Anovlar 21	4	50	30 months	Loss noticed after 28 months on pill

Two patients (Cases 1 and 2) stopped taking oral contraceptives 4 and 5 months before they noticed thinning of the hair, and one patient (Case 7) stopped the pill for 1 month, 4 months before the onset of symptoms. The first patient's hair thinning was still progressing after 2½ years. Although the thinning was not progressing, the second patient had not recovered after 1½ years. Four patients (Cases 3–6) lost hair when they changed the brand of oral contraceptive, but there is no obvious correlation with either the oestrogen or the progestogen content of the preparations used. (column 4 of Table 3). Patient 5 had also noticed hair loss after two of her three pregnancies.

TABLE 4. Cause of hair loss in eleven women taking oral contraceptives

Stopped pill	2			
		<	other possible cause	3
Still on pill	9		no other cause found	6

In four instances (Cases 8–11) the diffuse alopecia was not related to changing the brand of oral contraceptives. Patient 8 had been pregnant 6 months before the hair thinning started.

Of the nine patients (Cases 2–11) who were still taking oral contraceptives when the alopecia started a second factor may have contributed to the thinning of the hair of three (Cases 6–8). These are respectively, iron deficiency anaemia, pregnancy, and interruption of treatment. In the remaining six who were still taking the pill, no cause could be found for the alopecia in either the history or investigations. These factors are summarized in Table 4.

No characteristic changes were seen in the six scalp biopsies.

DISCUSSION

Cormia (1967) described five women who had diffuse hair loss which he attributed to oral contraceptives. The hair loss started in three patients when they stopped taking the pill. One had been continuously taking a very high dose of oestrogen for endometriosis when the hair loss started, and another was taking a sequential contraceptive.

Greenwald (1970) reported two patients who developed diffuse alopecia after stopping the pill and a further three who lost hair while still taking the pill.

Both studies lack controls and do not state whether other causes of thinning hair were excluded.

Dawber & Connor (1971) reported diffuse hair loss occurring in a woman 2 months after stopping an oral contraceptive. The hair fall ceased when she became pregnant but restarted 2 months after her delivery, and was still continuing after 4 months.

A patient may experience thinning of the scalp hair either because the hair is shed at an increased rate, or because hairs shed at the normal rate are not replaced normally. Thus, thinning hair is not necessarily associated with increased hair fall. The distinction is important because of the different mechanisms involved. It has been shown that the density of hair follicles in the normal scalp falls with age from $615/\text{cm}^2$ in the third decade to $485/\text{cm}^2$ in the fourth decade. A reduction of this magnitude may not be clinically apparent, but is clearly important in attempting to quantify pathological thinning of the scalp hair.

Pregnancy increases the percentage of hairs in the growing (anagen) phase of the follicular cycle. Several months after the pregnancy has ended hair may fall when the excess number of anagen hairs turn into resting (telogen) hairs (Pecoraro, Barman & Astore, 1969; Schiff, Pawtucket & Kern, 1963). Because oral contraceptives induce a state of pseudo-pregnancy, it has been suggested that hair loss occurring when medication is stopped may be analogous to post-partum hair loss. However, careful studies of the effect of oral contraceptives on the hair cycle have shown that the changes do not resemble those found in pregnancy (Zaun & Ruffing, 1970; Zaun & Gerber, 1969; Tronnier, 1970).

This study confirms that diffuse loss of scalp hair occurs in some women after they stop taking oral contraceptives.

Nine patients in the present study noticed thinning of the hair while still taking oral contraceptives. Was the pill responsible for the hair loss ? A possible alternative explanation was found in three patients leaving six in whom there were no factors other than the pill. Before concluding that the pill was responsible for the diffuse alopecia in these patients, it should be noted that no cause could be found in nine of the nineteen patients who had never taken oral contraceptives. When the number of patients with idiopathic thinning of the hair is compared for pill-takers and non-pill-takers, there is no significant statistical difference ($P > 0{\cdot}5$). Furthermore, there was no difference in the distribution or the severity of the alopecia in those who had taken oral contraceptives compared with those who had not.

No attempt has been made in this study to quantitate the proportion of anagen to telogen hairs, and this aspect would be worthy of further investigation. It may be difficult to avoid misleading or false results when counting the anagen/telogen ratio, unless it is appreciated that (1) it is fallacious to pluck hairs at random from the scalp (Van Scott, Reinertson & Steinmuller, 1957). (2) the anagen/telogen ratio may differ in different areas of the scalp, and (3) shampooing or briskly brushing the hair a day or two before the sampling alters the anagen/telogen ratio.

An epidemiological approach to the problem does not suggest that oral contraceptives are likely to cause hair loss. The popularity of oral contraceptives has greatly increased in the last decade. The number of women seeking advice for diffuse alopecia has not increased with the more widespread use of these drugs.

TABLE 5. Yearly incidence of women with diffuse hair loss at St John's Hospital for Disease of the Skin

Year	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970
No. of patients	142	136	140	166	137	158	108	144	126	70	88	108	126	178	160	203	153	132	129

Sulzberger, Witten, & Kopf, in 1960 reported an increasing incidence of diffuse alopecia in women in the United States of America, but the data for that report were collected before many women were taking oral contraceptives in America (World Health Organization, 1966).

The problem may be summarized thus:

(1) Some women who are taking oral contraceptives notice thinning of the hair.

(2) Some women who have never taken oral contraceptives notice thinning of the hair for no discernible reason.

(3) Are the two groups really the same and is taking the pill an irrelevant factor?

For further investigation it would be rewarding to study quantitatively the separate aspects of hair fall and hair replacement.

CONCLUSIONS

(1) Some women notice diffuse loss of scalp hair some 2 or 3 months after they stop taking oral contraceptives.

(2) Some women currently taking oral contraceptives also lose their hair, but there is at present no evidence to suggest that it is because they are taking oral contraceptives.

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Epidermal cell kinetics in lichen planus*

R. MARKS, M. BLACK AND E. WILSON JONES

Department of Pathology, St John's Hospital for Diseases of the Skin,
Lisle Street, Leicester Square, London, W.C.2

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SUMMARY

The epidermis in lichen planus presents an apparently paradoxical situation. There are on the one hand epidermal hypertrophy acanthosis and hypergranulosis, and on the other, destruction of the generative compartment with erosion of the basal layer. In order to understand how the two processes can co-exist, thirty-five lichen planus papules from twenty-five patients were injected intracutaneously with 10 μCi of tritiated thymidine. Sixteen of these lesions were removed after 50–60 min and nineteen were removed after longer intervals of up to 10 days. Autoradiographs prepared from biopsied lesions demonstrated that keratinocytes had migrated into the lichen planus lesion from the intact margins, replaced the eroded basal layer and repopulated the epidermis of the lesion. It is concluded that the changes observed in the epidermis seen in lichen planus can be interpreted as the result of a continuous process of (1) erosion and (2) repopulation in an attempt at healing of the wounded area.

Lichen planus (LP) is a common dermatosis, yet we have little insight into the mechanisms responsible for producing the characteristic itchy papular lesions. It has been assumed that just as psoriasis is characterized by a 'high output' state of epidermal proliferation, lichen planus represents a condition of 'low output' and it has been suggested by Pinkus & Mehregan (1969) that there is a low rate of epidermal cell renewal in LP. If there was a low rate of epidermal cell turnover in LP then there would appear to be a paradox as histological examination reveals on the one hand an erosive process affecting the basal generative compartment, and on the other hypertrophy of the upper epidermis with acanthosis, hypergranulosis and hyperkeratosis. In the present paper we have tried to characterize the rate of epidermal cell proliferation in an attempt at understanding the histogenesis of the epidermal changes seen in this disease.

METHODS

Thirty-five papular lesions from twenty-five patients over 40 years of age were injected intracutaneously with 10 μCi of tritiated thymidine (Radiochemicals Amersham sp. activity 26 Ci/mmole) in 0·1 ml of N saline. The injected sites were then removed at intervals of up to 10 days subsequently as indicated in Table 1.

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* Based on a paper read at the combined meeting of the Society for Investigative Dermatology and the European Society for Dermatological Research, Amsterdam, 19 May 1972.

These biopsies were fixed in 10% formol saline, dehydrated, wax embedded, sectioned at 5 μm, dewaxed, taken to water and then dipped in nuclear emulsion (Ilford K2) according to the method of Joftes & Warren (1955). They were then developed in Kodak D19 developer and stained with haematoxylin and eosin. Three of the sixteen biopsies removed 50–60 min subsequent to injection with tritiated thymidine were embedded at right angles to the usual vertical plane of orientation and sectioned horizontally throughout the entire tissue. Autoradiographs were prepared from all the sections cut in the horizontal plane.

TABLE I

No. of specimens	Interval between injection and removal
16	50–60 min
1	1 day
4	2 days
2	3 days
2	4 days
3	5 days
1	6 days
4	7 days
–	8 days
1	9 days
1	10 days

RESULTS

(1) *Autoradiographs prepared from biopsies removed after injection with tritiated thymidine* 50–60 *min previously*

As expected, in many areas there was little labelling of the cells in the basal zones. The cells in these unlabelled areas were abnormal, large and showed marked eosinophilia. However, amongst these cells there were smaller, basophilic epidermal cells that were labelled and which occurred in groups and columns (Fig. 1). These cells do not seem to have been described before in LP and it seems that the labelling drew our attention to them. These cells were approximately one-quarter to one-third the size of the neighbouring epidermal cells and varied in shape from spindle-like or fusiform to spherical. Often several consecutive 'spindle-shaped' cells showed labelling, giving the impression of a line of labelled cells at the base of the epidermis (Fig. 2). The grouping and clustering of labelled cells was particularly well seen in the specimens that were cut in the horizontal plane (Fig. 3a and b). Another feature observed was the grouping of labelled cells at the sides of epidermal ridges containing intra-epidermal sweat ducts (Fig. 4). On some occasions it seemed that the spindle-shaped type of cell had tracked between colloid bodies and the viable epidermis (Fig. 5). Labelling indices were determined by counting the number of labelled basal and suprabasal cells per 1000 basal cells. These values were determined both for the epidermis of the papule itself and for the adjacent uninvolved epidermis. These 'uninvolved areas' had no apparent basal cell damage although on occasions some pigmentary incontinence and slight inflammatory cell infiltrate might have been present. It was possible to obtain a labelling index for the uninvolved epidermis in six instances (three specimens cut horizontally were excluded, seven specimens did not have sufficient 'uninvolved epidermis' for evaluation) and for the involved epidermis in ten instances (three specimens cut horizontally were excluded, three specimens were unevenly labelled and were excluded). The labelling indices and the standard errors of the means are given below expressed as percentages.

Uninvolved epidermis	17·2±6·3	(×1·4 after correction by	= 24·1
Involved epidermis	15·4±3·2	Weinstein & Frost, 1969)	= 21·6

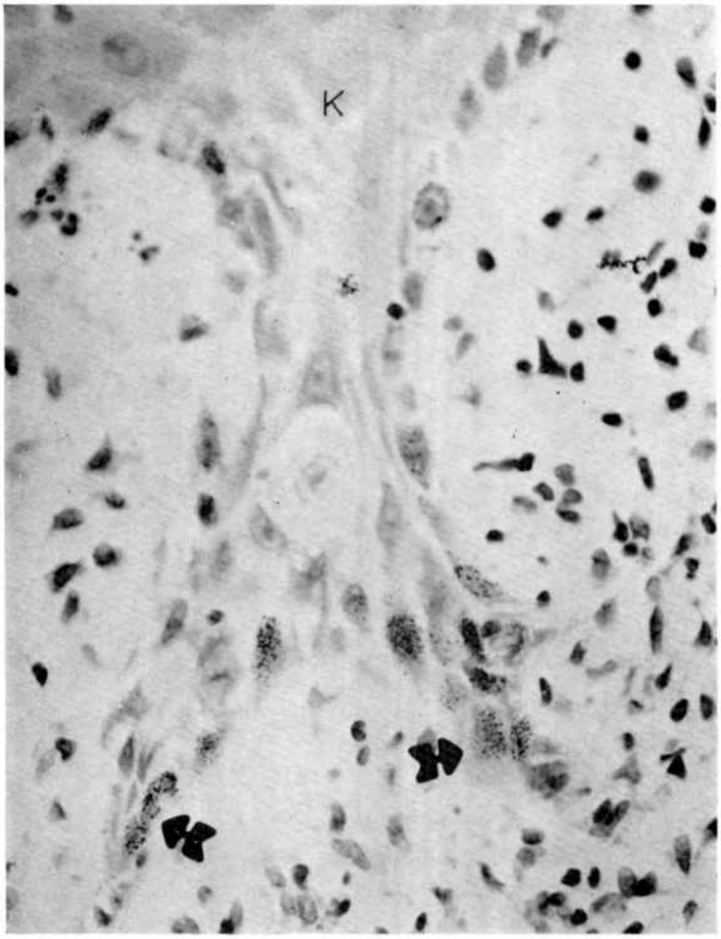

FIGURE 1. Autoradiograph from lichen planus papule injected with tritated thymidine 50 min previously. Oval and fusiform labelled cells are seen (arrowed) around larger and paler keratinocytes (K) (H & E, ×200).

(2) *Autoradiographs from biopsies removed* 1–10 *days after intracutaneous tritiated thymidine injection*

The highest point that the labelled cells had reached in the epidermis in each biopsy has been charted in Fig. 6. It can be seen that the rate of travel through the Malpighian layer varied in different specimens. In some sections the granular cell layer was reached within 3 days while in others the granular cell layer had not been reached at 7 days. Only in the 9 and 10 day specimens were labelled cells detected in the granular cell layer. In general, transit through the Malpighian layer seemed more rapid than through the granular cell layer. The same clustering of labelled cells around intra-epidermal sweat ducts was seen in the group of 'interval' specimens as in the specimens removed at 50–60 min.

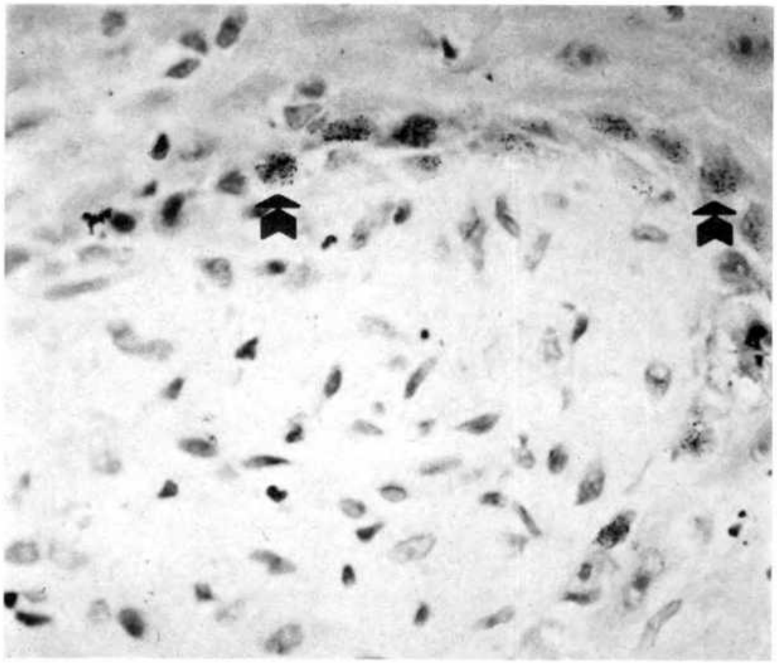

FIGURE 2. Similar autoradiograph to Fig. 1, showing a column of labelled cells (arrowed) at the base of the epidermis (H & E, × 200).

However, the labelled cells in these specimens were nearer to the lumen than in the earlier biopsies (Fig. 7—compare Fig. 4).

It was also noted that some of the swollen eosinophilic cells in the basal zone were labelled in the 2 and 3 days specimens.

DISCUSSION

Despite the profound epidermal changes that are found in the papules of LP, there have been very few investigations concerning the pathogenesis of these lesions. Lever (1967) and Pinkus & Mehregan (1969) have suggested that epidermal cell turnover is decreased in LP although there is little experimental evidence to support this concept. In one investigation, Hendren & Pinkus (1954) did show that after mitotic suppression of the epidermis by Thorium-X applications the epidermal cells enlarged and the proportion of maturing cells increased. One other study (El-Labban, Lucas & Kramer, 1971) investigated the mitotic index in the buccal mucosal lesions of LP and found that this quantity was lower than that found in leukoplakic lesions. In addition, it has been shown histochemically that there is a drop in the respiratory enzyme activity within the involved epidermis of the LP papule (Black & Wilson Jones, 1972).

In essence, the present investigation has demonstrated the presence of a previously unrecognized type of epidermal cell in the basal regions of the LP papule. These cells were observed to occur in groups, to be smaller and more basophilic than the neighbouring epidermal cells and to vary in shape from spindle-shaped or fusiform to spherical. It was this type of cell exclusively that became labelled after short-term exposure to tritiated thymidine and it was the labelling that drew our attention to them. They were especially well seen in the horizontally cut sections and in these preparations it was

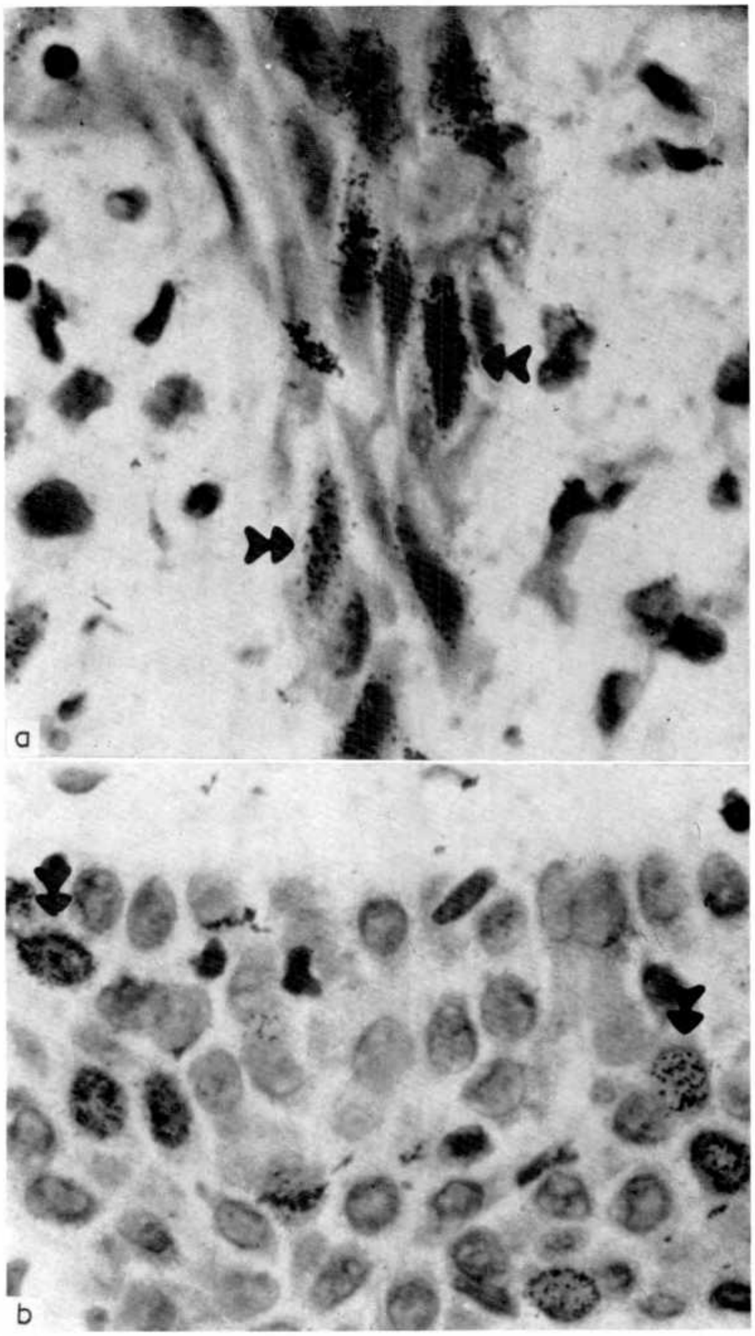

FIGURE 3. (a) Autoradiograph prepared from a horizontal section through the basal region of a lichen planus lesion. A column of labelled spindle-shaped and fusiform cells are seen (arrowed) (H & E, × 400). (b) Similar preparation to (a) but illustrating the variable morphology of these small labelled keratinocytes (arrowed) (H & E, × 400).

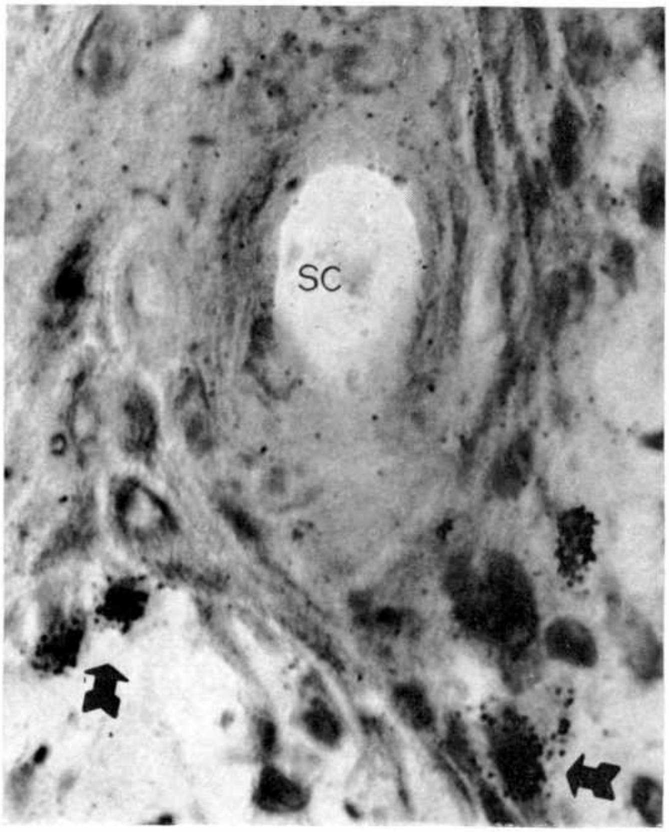

FIGURE 4. In this autoradiograph also prepared from a lichen planus lesion injected 50 min previously with tritiated thymidine there are labelled cells (arrowed) around an intra-epidermal sweat coil (SC) at the base of the epidermis (H & E, × 400).

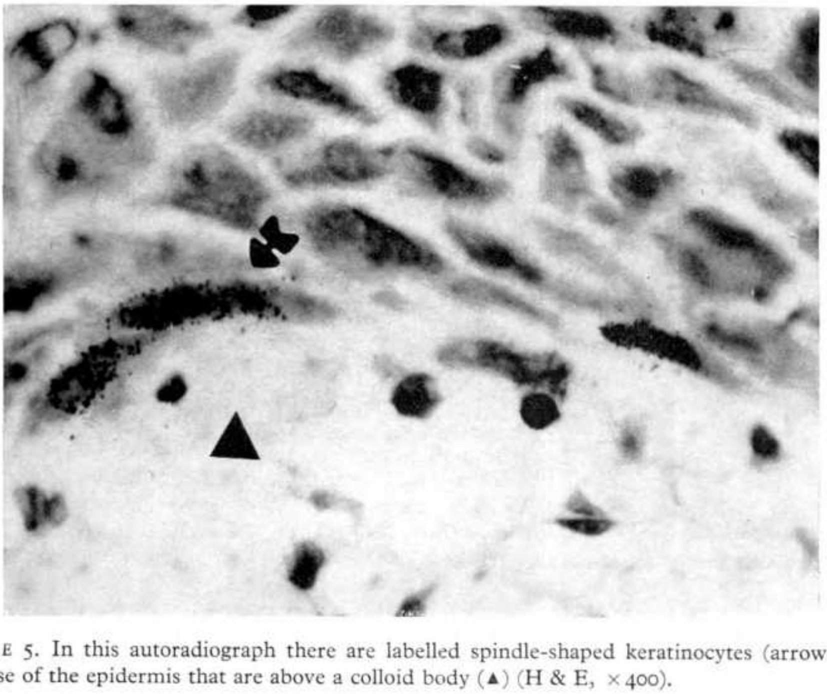

FIGURE 5. In this autoradiograph there are labelled spindle-shaped keratinocytes (arrowed) at the base of the epidermis that are above a colloid body (▲) (H & E, × 400).

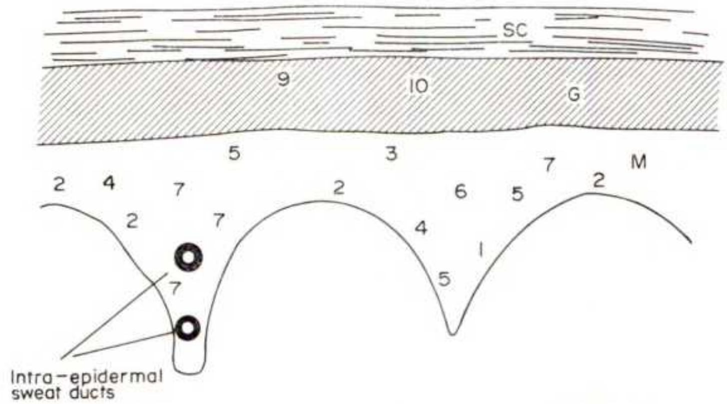

FIGURE 6. Diagram to show the highest point reached by labelled cells at the various time intervals in days between injection with tritiated thymidine and biopsy of the injected site.

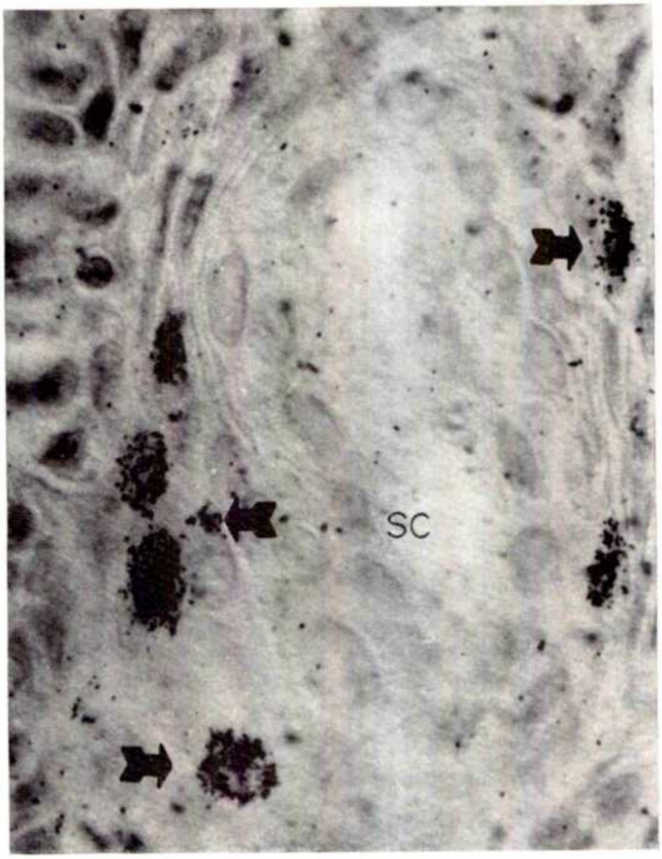

FIGURE 7. Autoradiograph prepared from lichen planus papule injected with tritiated thymidine 7 days previously showing labelled cells (arrowed) grouped around an intra-epidermal sweat coil (SC) (H & E, ×400).

evident that clusters and linearly arranged columns of these cells labelled synchronously. We interpret these findings to indicate that epidermal cells have migrated from uninvolved areas to make good the deficit produced by the erosive process of LP. The considerably increased labelling index (of perhaps four times the normal) for the unaffected epidermis at the margins of the papule of 17·2±6·3% (corrected 24·1%) is also consistent with this interpretation and supports the concept of keratinocytes being 'supplied' from these areas. There was in addition an increased labelling index of 15·4±3·2% (corrected 21·6%) for the epidermis of the LP papule but as there were no 'normal' basal cells in these involved areas, the validity of the value for the LP papule is doubtful.

The autoradiographs prepared from biopsies removed 1–10 days after intracutaneous injection of tritiated thymidine show that the rate of transit through the Malpighian layer varies considerably from papule to papule. The granular cell layer was reached in some instances in 3 days yet had not been reached in 7 days in others. In the specimens removed at 9 and 10 days there were a few labelled cells within the granular cell layer and it seemed that while travel through the Malpighian layer could be quite speedy, the passage through the granular cell layer was a slower affair. (The rate of transit through the normal viable epidermis is approximately 14 days.)

An additional feature of interest was the apparent preferential labelling of cells at the base of the epidermis around intra-epidermal sweat ducts in the specimens removed 50–60 min after exposure to tritiated thymidine (Fig. 4). The same clustering of labelled cells around intra-epidermal sweat coils occurred in the 'interval' biopsies, but appeared nearer the lumen of the duct (Fig. 7). This apparent inward movement of some labelled cells has recently been shown to occur in the normal sweat duct by Christophers & Plewig (1972).

In the healing of a cutaneous wound, epidermal cells actively migrate in the first instance from the intact margins into the breach and later cell division occurs to make good the cell loss (Viziam, Matoltsy & Mescon, 1964). The destruction of the basal layer in LP may be regarded as equivalent to a 'microwound' and our observations support the concept of an attempt at wound healing by the migration of epidermal cells from either side and probably from the epithelium of the intra-epidermal sweat coils as well. The finding of occasional epidermal cells that are labelled, but which are swollen and eosinophilic and which remain in the basal regions in the 2 and 3 day interval biopsies, and the varying rate of travel through the Malpighian layer suggest an explanation for the persistence of the LP papule. It seems that the erosive process is a continuous one and that there is a continual battle between cell destruction and repopulation; whether healing or further basal cell destruction takes place or the status quo is maintained must depend on the balance struck between the two processes. If the LP papule is regarded as an epithelial wound with constant erosion on the one hand and repopulation on the other, then the observed changes within the epidermis can be understood. During the healing of a wound all compartments of the epidermis may show hypertrophy. If at the same time the basal layer of the healing area is constantly damaged, there will appear to be disproportionate acanthosis and hypergranulosis.

Despite many previous investigations we are no nearer to an understanding of the ultimate cause of LP (Black, 1972). Our studies, however, have clarified one issue, in that it is clear that the 'paradox' of hypertrophy in the apparent absence of an intact generative compartment in LP is at least partially explicable on the basis of repopulation of the involved epidermis by the migration of epithelial cells from the margins.

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Pharmacology and Treatment

Effect of non-steroid anti-inflammatory drugs on prostaglandin biosynthesis by skin

MALCOLM W. GREAVES AND WENDY McDONALD-GIBSON

University Department of Dermatology, Newcastle-upon-Tyne, 1

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SUMMARY

We report inhibition of *in vitro* synthesis of prostaglandins by skin in the presence of aspirin and indomethacin. In the presence of aspirin significant inhibition of enzymic synthesis of prostaglandin $F_{2\alpha}$, but not prostaglandin E_2, was demonstrated. Indomethacin also caused a significant inhibition of synthesis of prostaglandin $F_{2\alpha}$, but had no effect on prostaglandin E_2 synthesis. Since there is increasing evidence that prostaglandins mediate some types of inflammation, the possibility arises that inhibition of prostaglandin biosynthesis explains at least part of the anti-inflammatory activity of these drugs.

There is increasing evidence that prostaglandins are important mediators of cutaneous inflammation. In earlier work we have shown that prostaglandins are the most consistently recovered vasoactive agents in cutaneous inflammation due to contact eczema and exposure to ultraviolet radiation (Greaves & Søndergaard, 1970; Greaves, Søndergaard & McDonald-Gibson, 1971) and similar findings have been reported in inflamed human skin following thermal burns (Angaard, Arturson & Jonsson, 1970). Prostaglandins are also highly vasoactive in human skin (Søndergaard & Greaves, 1971).

Recently Vane (1971) demonstrated *in vitro* biosynthesis of prostaglandins by guinea-pig lung tissue, and reported that this reaction could be suppressed by some anti-inflammatory drugs.

Prostaglandin biosynthesis by skin *in vitro* was first demonstrated by one of us (Jessup *et al.*, 1970) and more recently by Van Dorp (1971) and Greaves & McDonald-Gibson (1972). In this paper we report the effects of the anti-inflammatory drugs aspirin and indomethacin on the rate of biosynthesis of prostaglandins E_2 and $F_{2\alpha}$ by mammalian skin.

METHODS

Shaved abdominal skin was removed from freshly killed female Wistar Porton albino rats (wt approx. 200 g). After removing subcutaneous fat and blood vessels the skin was weighed and divided into small fragments with scissors and added to phosphate buffer (pH 7·4) containing hydroquinone (0·275 mmol) and glutathione (0·325 mmol) (Foss *et al.*, 1971). The skin was then homogenized using a Polytron tissue homogenizer, and bovine serum albumin (final concentration 5 mg/ml) was added to the homogenate. The homogenate was then incubated aerobically with excess arachidonic acid substrate containing tritium-labelled arachidonic acid tracer (New England Nuclear Inc.) in a shaking water bath at 37°C. The final concentration of added arachidonic acid was 12·5 μg/ml and the reaction volume 2 ml in all experiments. The reaction was terminated after 40 min by adding 10 ml ice-cold ethanol. After 4 h the supernatant was recovered and the residue washed with ice-cold ethanol. The washings and corresponding supernatants were combined and stored deep-frozen prior to acidic lipid extraction using petroleum ether and diethyl ether followed by thin-layer chromatography as described by Greaves & McDonald-Gibson (1972). Prostaglandin E_2 and $F_{2\alpha}$ activity was determined by elution of zones of the chromatoplate corresponding to simultaneously developed standard prostaglandin E_2 and $F_{2\alpha}$ followed by determination of radioactivity in these eluates using a Packard Tri-carb Liquid Scintillation counter. A comparison of concentrations of prostaglandins E_2 and $F_{2\alpha}$ in eluates from the prostaglandin E_2 and $F_{2\alpha}$ zones of the chromatoplate, determined by measurement of radioactivity or by bioassay using an isolated rat uterus preparation, gave identical results, thus confirming that the recovered activity was due to prostaglandin E_2 or $F_{2\alpha}$. The mean recoveries of PGE_2 and $PGF_{2\alpha}$ added to the skin homogenates exceeded 90%. Under the same conditions variation in rate of prostaglandin E_2 and $F_{2\alpha}$ synthesis between aliquots of the same skin sample was less than 10%.

The effect of aspirin and indomethacin on biosynthesis of prostaglandin E_2 and $F_{2\alpha}$ was studied by adding each drug to the reaction mixture immediately before incubation. All experiments included controls in which arachidonic acid and other reagents were incubated with buffer alone before addition of homogenate, extraction and thin-layer chromatography.

RESULTS

In twenty-nine rats the mean total prostaglandin biosynthesis in 40 min was 1·28 μg/g wet weight. Of this 0·86 was prostaglandin E_2 and 0·42μg was prostaglandin $F_{2\alpha}$.

TABLE 1. Aspirin on prostaglandin synthesis

	Prostaglandin synthesis (ng/g wet wt/40 min)	
	Aspirin concentration (mmol)	
	0·56	1·68
PGE_2	765 ± 188 (650 ± 144)	2286 ± 451 (2235 ± 114)
$PGF_{2\alpha}$	262 ± 52 (386 ± 57)	377 ± 142 (556 ± 141)
	$P < 0·05$	$P = 0·025$

Values represent mean of six experiments ± SEM. Figures in brackets refer to control values. PG, prostaglandin.

Aspirin

The results are summarized in Table 1. In six experiments aspirin 0·56 mmol produced a mean inhibition of prostaglandin $F_{2\alpha}$ synthesis of 32% ± 13 SEM and this was statistically significant ($P = < 0·05$). A three-fold increase in the concentration of aspirin (1·68 mmol) in a further six experiments did not significantly increase the inhibition of prostaglandin $F_{2\alpha}$ synthesis. Neither concentration of aspirin caused significant inhibition of prostaglandin E_2 or total prostaglandin synthesis.

Indomethacin

Indomethacin caused inhibition of prostaglandin $F_{2\alpha}$ synthesis (Table 2). At a concentration of 0·28

TABLE 2. Indomethacin on prostaglandin synthesis

	Prostaglandin synthesis (ng/g wet wt/40 min)	
	Indomethacin concentration (mmol)	
	0·28	0·74
PGE_2	535 ± 121 (650 ± 144)	2125 ± 345 (2235 ± 114)
$PGF_{2\alpha}$	245 ± 79 (386 ± 57)	251 ± 151 (556 ± 141)
	$P<0{\cdot}05$	$P<0{\cdot}05$

Values represent mean of six experiments ± SEM. Figures in brackets refer to control values. PG, prostaglandin.

mmol inhibition was 37% ± 20 ($P<0{\cdot}05$). However, when in six further experiments the concentration of indomethacin was increased to 0·74 mmol, inhibition was 55·0% ± 4·0 SEM (Table 2). Neither concentration caused significant inhibition of either prostaglandin E_2 or total prostaglandin synthesis.

DISCUSSION

The present results show that synthesis of prostaglandins by skin can be inhibited by aspirin or indomethacin. That the drugs cause inhibition of prostaglandin $F_{2\alpha}$ synthesis is in agreement with results recently reported by Vane (1971) who found that aspirin and indomethacin both caused significant inhibition of biosynthesis of prostaglandins E_2 and $F_{2\alpha}$ using guinea-pig lung as a source of prostaglandin synthetases. His results differ from ours since his prostaglandin synthetase preparation was at least ten times more sensitive to indomethacin and about twice as sensitive to aspirin as ours. However, a greater sensitivity of his preparation may be due to differences in technique since Vane used a cell-free homogenate as a source of synthetase whereas we used an unseparated homogenate of skin. Like Vane we have demonstrated that the inhibitory effect of indomethacin on prostaglandin $F_{2\alpha}$ synthesis is dose related, and that prostaglandin synthetase is more sensitive to indomethacin than aspirin. We were, however, unable to demonstrate that the inhibition of prostaglandin $F_{2\alpha}$ by aspirin was dose related. The reason for this is uncertain and raises the possibility that inhibition by aspirin may involve more than one mechanism. Recent evidence suggests that one site of action of these drugs may be the early, oxygen-dependent activation stage of biosynthesis (Smith & Lands, 1971). We were unable to demonstrate inhibition of prostaglandin E_2 synthesis by aspirin or indomethacin. It is of interest that these drugs were also much less inhibitory on prostaglandin E_2 synthesis in Vane's experiments. The relevance of these findings to the anti-inflammatory actions of the two drugs is uncertain. The vasoactive potency of prostaglandin $F_{2\alpha}$ is low compared with the 'E' prostaglandins (Crunkhorn & Willis, 1971). Furthermore, aspirin and indomethacin given by the oral route are not notably effective in mitigating cutaneous inflammation. The present results do however suggest that *in vitro* prostaglandin biosynthesis in skin may be an important model for investigation of the pharmacology of anti-inflammatory drugs.

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Blockade by polyphloretin phosphate of the prostaglandin E_1-induced human cutaneous reaction

JØRGEN SØNDERGAARD AND HANS PAULLI JØRGENSEN

Department of Dermatology, University of Copenhagen,
Rigshospital, 2100 Copenhagen, Denmark

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SUMMARY

The effect of polyphloretin phosphate (PPP) on the human cutaneous reaction induced by prostaglandin E_1 (PGE_1) has been studied in thirty subjects. Pre-treatment of the skin with three intradermal injections of 100 μg PPP reduced by 37% the magnitude of the erythema induced by intradermal injection of 1 μg PGE_1. The PGE_1-induced weal, which is probably mainly due to PGE_1-induced endogenous histamine release, was not reduced. The specific nature of the PG-blocking activity of PPP was supported, since PPP did not block the erythema and weal induced by intradermal injection of histamine and bradykinin. Intravenous injection of 100 mg PPP did not reduce the erythema due to intradermal injection of PGE_1 probably because of too low tissue concentration of PPP.

Polyphloretin phosphate (PPP), a mixture of phosphorylated phloretin polymers and a potent inhibitor of alkaline phosphatase and hyaluronidase (Diczfalusy *et al.*, 1953), antagonizes the *in vitro* action of prostaglandins (PG) on isolated organs of several animal species (Eakins & Karim, 1970; Eakins, Karim & Miller, 1970). More recently, PPP has been shown to block the *in vitro* contractile effects of $PGF_{2\alpha}$ in human bronchi (Mathé, Strandberg & Åström, 1971). In addition, PPP antagonizes some *in vivo* effects of exogeneous prostaglandin (Bethel & Eakins, 1971; Villanueva *et al.*, 1971). Previously it has been shown that intradermal injection of prostaglandin E_1 in human subjects causes a persistent dusky erythema and a well-defined weal (Søndergaard & Greaves, 1971) and PGE_1 is released together with other prostaglandins in human inflamed skin (Søndergaard & Greaves, 1970; Greaves, Søndergaard & McDonald-Gibson, 1971). In the present study we have examined whether PPP could modify the cutaneous reaction induced by PGE_1.

MATERIALS AND METHODS

Patients

Thirty volunteer subjects were studied. These were all patients with localized skin conditions such as alopecia, bacterial infections, neoplasms, warts and leg ulcers. The arms were uninvolved in all cases.

Pretreatment with PPP

Intradermal injections of PPP were given to fifteen subjects. The injections were performed on the flexor aspect of the forearm and a volume of 0·1 ml was injected in each case, using a 1 ml tuberculin syringe and a needle of external diameter of 0·5 mm. Each patient received three injections of 100 μg PPP at the same site on the forearm at hourly intervals. One hour after the third injection 1 μg PGE_1 was given at the same site as the PPP injections. After 20 and 60 min the resultant weal and erythema were traced on transparent paper and the areas were measured planimetrically.

In ten of the fifteen patients, series of three injections of PPP performed on three different sites of the forearm were followed by intradermal injection of 1 μg PGE_1, 10 μg histamine or 1 μg bradykinin. The opposite arm served as control and three injections of 0·154 M NaCl were given prior to the PGE_1 histamine or bradykinin injections.

Intravenous injections of PPP

Intradermal injections of PGE_1 (1 μg), histamine (10 μg) and bradykinin (1 μg) were performed in fifteen subjects and the resultant weal and erythema registered 20 and 60 min later. PPP, 100 mg dissolved in 10 ml 0·154 M NaCl was then slowly injected intravenously (1 ml/min) and the blood pressure and possible clinical side effects recorded. One hour later injections of PGE_1 (1 μg), histamine (10 μg) and bradykinin (1 μg) were performed symmetrically on the opposite arm and the areas registered 20 and 60 min later.

TABLE 1. Effect of intracutaneous injection of polyphloretin phosphate on skin reactions induced by prostaglandin E_1, histamine and bradykinin

		20 min		60 min	
Injection	No. of patients	Weal (mm^2)	Erythema (mm^2)	Weal (mm^2)	Erythema (mm^2)
PGE_1	15	104 ± 14	597* ± 95	41 ± 14	579* ± 75
Control	15	117 ± 13	947 ± 112	52 ± 18	922 ± 84
Histamine	10	257 ± 18	2439 ± 277	183 ± 25	1479 ± 205
Control	10	265 ± 19	2629 ± 316	246 ± 41	1677 ± 193
Bradykinin	10	152 ± 12	0	59 ± 21	0
Control	10	141 ± 8	0	44 ± 20	0

* Significantly different from the controls at the 5% level of probability.

RESULTS

Initial studies revealed that intradermal injection of 500 μg PPP caused a red irritant reaction whereas 100 μg caused no visible skin reaction. Therefore, 100 μg PPP was used for the intradermal injections. The skin was pretreated with three intradermal injections of PPP since preliminary experiments revealed that PPP did not reduce the PGE_1 reaction if PPP (100 μg) was injected together with 1 μg PGE_1.

A significant reduction of the erythema was observed in fifteen patients receiving three intradermal injections of PPP prior to PGE_1 (Table 1). The reduction, measured 60 min after PGE_1 injection, amounted to 37±11% ($P<0{\cdot}05$) compared with NaCl-pretreated skin. Pretreatment with PPP did not reduce the weal induced by PGE_1 and no reduction of the area of the weal and erythema due to histamine and bradykinin was observed (Table 1).

Intravenous injection of 100 mg PPP caused no significant diminution of the area of the PGE_1-

induced weal (7% ± 6, $P > 0{\cdot}05$) or erythema (14% ± 9, $P > 0{\cdot}05$), as measured 60 min after the PGE_1 injection. In the same experiments intravenous injection of 100 mg PPP failed to reduce the area of the weal and erythema due to histamine and bradykinin.

The arterial blood pressure, measured before, during and after the intravenous injection of PPP was unaltered and no clinical side-effects were noticed.

DISCUSSION

Polyphloretin phosphate counteracts some of the *in vitro* effects of prostaglandins (Eakins & Karim, 1970; Mathé, *et al.*, 1971). PPP, however, has not been tested previously *in vivo* using human skin. In this study PPP exerted a specific antagonistic effect on the human cutaneous reaction induced by intradermal injection of PGE_1. However, a significant inhibitory effect was only registered when the skin was pretreated with three injections of 100 μg PPP, but not when 100 mg PPP was intravenously injected. PPP probably acts by competitive antagonism (Eakins, Miller & Karim, 1971; Mathé *et al.*, 1971). The failure of PPP to block the PGE_1 reaction when intravenously injected is probably due to too low tissue concentration.

A specific PG-blocking activity of PPP has been postulated (Eakins *et al.*, 1970). The results of the present experiments lend support to this view since only the PGE_1-induced erythema, but not the reaction due to histamine and bradykinin could be blocked by PPP. PPP did not reduce the area of the PGE_1-induced weal, probably because the weal is mainly due to endogenous histamine release brought about by PGE_1 (Søndergaard & Greaves, 1971).

A possible inhibitory effect of PPP on cutaneous hyaluronidase cannot be excluded in the present experiments. In other experimental conditions, however, it has been shown that PPP exerts its prostaglandin-blocking effects through mechanisms unrelated to its ability to inhibit hyaluronidase (Eakins *et al.*, 1971).

The finding that pretreatment of the skin with PPP causes a suppression of the cutaneous PGE_1 reaction is particularly interesting in the view of the indications that PGE_1 may be involved in sustained cutaneous inflammation in man (Søndergaard & Greaves, 1970; Greaves *et al.*, 1971).

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Intralesional treatment of alopecia areata with triamcinolone acetonide by jet injector

E.ABELL AND D.D.MUNRO

St John's Hospital for Diseases of the Skin, Lisle Street, London, W.C.2, and Departments of Dermatology,
St Bartholomew's Hospital, London, E.C.1 and Edgware General Hospital, Middlesex

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SUMMARY

Eighty-four patients with alopecia areata of varying degree have been treated by intralesional injections of triamcinolone acetonide, using a needleless injector (Porto-Jet). Results indicate that, in limited alopecia, a high proportion will produce a rapid regrowth of hair. In established alopecia totalis, lasting hair growth is exceptional.

Treatment of alopecia areata by intradermal corticosteroid injections has been practised for many years. Kalkoff & Macher (1958) appear to be the first to report a series using hydrocortisone. Subsequently, Orentreich *et al.* (1960) and Gombiner & Malkinson (1961) reported the use of prednisone and triamcinolone, and Porter & Burton (1971) used triamcinolone acetonide and hexacetonide. With the development of jet injection apparatuses, more widespread use of local steroid injections could be undertaken. Moynahan & Bowyer (1965) and Verbov & Abell (1970) reported initial results of such techniques in a number of conditions, including alopecia areata. This report gives details of treatment and results in a large series of alopecia areata cases.

MATERIAL AND METHOD

Eighty-four patients with alopecia areata, varying in degree from a single lesion to the universal form of the disease, were treated by intradermal injections of triamcinolone acetonide (TA) at a concentration of 5 mg/ml, using the 'Porto Jet' needleless injector, as described by Verbov & Abell (1970). Injections of 0·1 ml were given at weekly or 2-weekly intervals on three occasions, and the patients subsequently examined for hair growth 6 and 12 weeks after completion of treatment. The number of injections given was determined by the extent of the alopecia and the frequency of treatment, the maximum being 60 (30 mg TA) when given at 2-weekly intervals, the average being 28 (14 mg TA).

In fifteen patients exact or comparable numbers of injections of isotonic sterile normal saline were given coincidentally with the triamcinolone therapy, after first flushing the apparatus free of the steroid.

At follow-up, satisfactory regrowth was assessed only if predominantly pigmented terminal hair was found at the injection sites in significant quantity, careful examination for similar evidence of regrowth also being made at untreated and saline-treated sites. Regrowth following treatment occurred

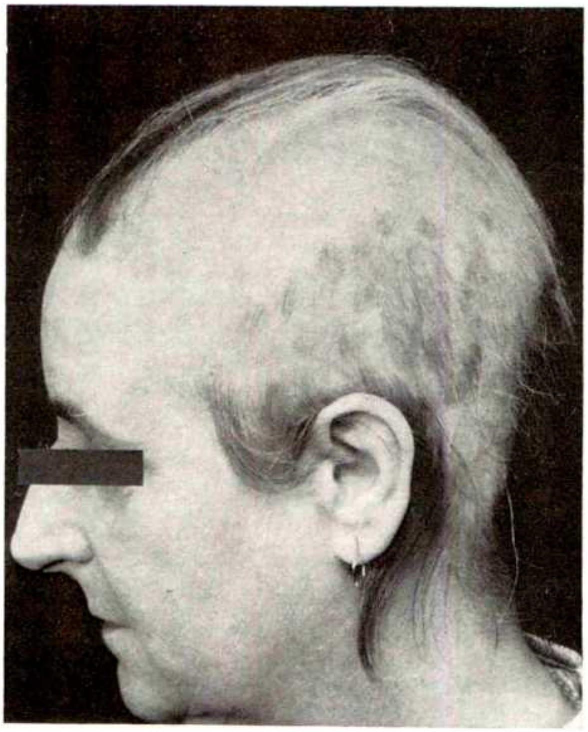

FIGURE 1. Tufted hair regrowth 6 weeks after treatment.

in tufts corresponding to the injection sites within 4–6 weeks, and was often accompanied by evidence of minimal, transient atrophy (Fig. 1).

RESULTS

Table 1 demonstrates the results, both as a whole and after subdivision into limited alopecia and alopecia totalis (and universalis).

A substantial proportion of both groups loses hair after regrowth at 6 weeks. This is more marked in the latter group.

Forty-seven (71%) of the limited alopecia patients maintained hair regrowth at 12 weeks, as

TABLE 1

	Nos.	Regrowth at 6 weeks		Regrowth at 12 weeks		Fall after initial growth		Growth associated with spontaneous recovery	
Total	84	72	86%	52	62%	20	24%	12	17%
Alopecia areata	66	61	92%	47	71%	14	21%	8	12%
Alopecia totalis	18	11	61%	5	28%	6	33%	4	22%
Controls	15	1	7%	1	7%	0		1	7%
Beard area	7	6	86%	4	57%	2	29%	0	—
Eyebrows	5	3	60%	1	20%	2	40%	0	—

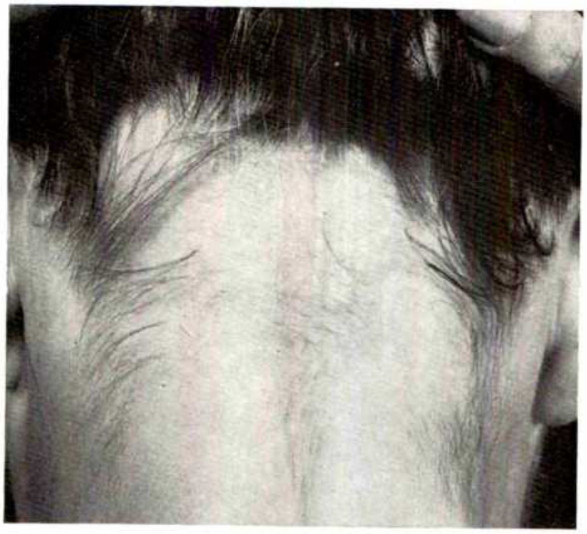

FIGURE 2. Occipital alopecia of 1 year's duration.

opposed to five (28%) of the totalis group. By making allowances for those showing spontaneous regrowth during the same period, these figures are further reduced to thirty-nine (60%) and one (6%) respectively in whom there appears to have been a definite therapeutic response.

A small number of patients were treated for alopecia localized to the beard area, and for alopecia totalis also involving the eyebrows. These results are also presented in Table 1. More than half those with beard alopecia had a satisfactory outcome.

Complications

Haemorrhage at the puncture site was a common occurrence, but could be easily controlled by direct pressure. Sepsis was not seen at any of the 7000 injection sites.

Minimal atrophy frequently occurred, but did not persist after the 12-week follow-up, except in

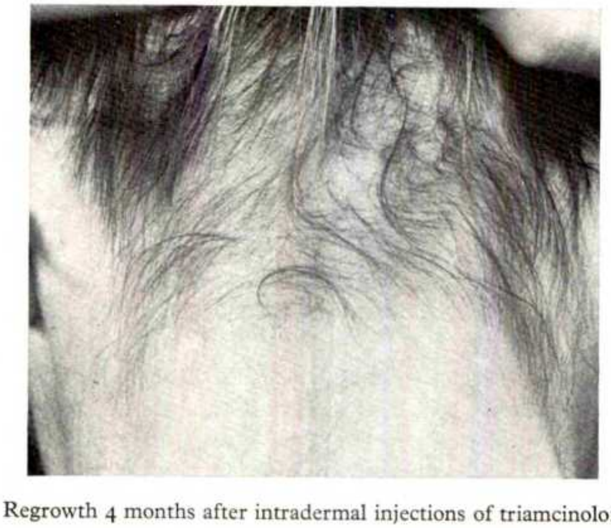

FIGURE 3. Regrowth 4 months after intradermal injections of triamcinolone acetonide.

two cases, and in these instances resolution was complete within a further 8 weeks. Only slight discomfort was experienced on injection, but more troublesome pain occurred in two patients. No adequate explanation could be found.

In one patient plasma cortisol values were monitored by 9.00 a.m. samples. The pretreatment level of 14·8 μg/100 ml was depressed to 2·7 μg/100 ml the day following a dosage of 22·5 mg of triamcinolone acetonide. The resting level was regained by day 3 and remained normal over the next 10 days.

DISCUSSION

Because of the variable natural history of alopecia areata the results of any treatment must be viewed with circumspection. The needleless injector does not lend itself easily to double blind methods of evaluating therapy. The incidence of spontaneous recovery within the period of observation was 20%.

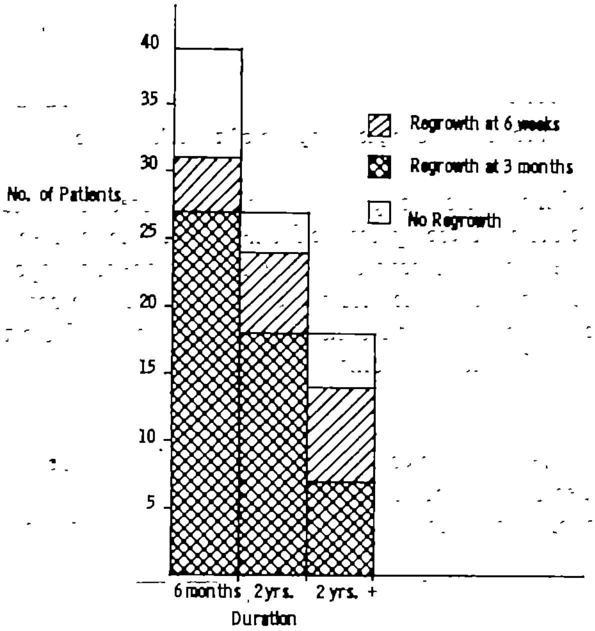

FIGURE 4. Results of treatment by duration of condition.

Little difficulty usually arises in discerning regrowth due to treatment from that occurring spontaneously. The possibility of a systemic effect cannot be ruled out, as evidenced by the plasma cortisol suppression in the case cited.

To be of value, treatment must produce lasting regrowth which is of cosmetic use (Figs. 2 and 3). The majority of patients were observed for longer than the trial period, and additional injections were given as necessary after 3 months. Retreatment was attempted in two cases of alopecia totalis, with persistently poor results.

The response to injection of the eyebrows in five cases of total alopecia reflects the same poor result as for the scalp. Localized beard alopecia, from the small numbers treated, probably gives a similar response to localized alopecia of the scalp.

In all previous series, save that of Gombiner & Malkinson (1961, report of four cases), hair fall after regrowth is recorded as particularly associated with extensive alopecia, and the latter is generally held to be a poor prognostic sign, especially in the prepubertal group of cases. Despite this, of the eighteen alopecia totalis cases in this series, six ultimately made a spontaneous recovery, three of four where the condition had existed for less than 6 months, and three of seven with a duration of 6–24 months. No permanent regrowth was seen in the seven cases established for over 2 years. Figure 4 shows that the response to treatment is poor when the condition has persisted for over 2 years, and these recalcitrant cases adversely affect the figures for the whole group. None of the three cases of prepubertal total alopecia produced lasting regrowth. When alopecia is in a rapidly progressive phase of the disease, intralesional injections fail. In the nine cases in which this happened not a single case maintained hair regrowth at 12 weeks, despite the fact that three are known to have resolved within the next year.

The usefulness of this form of treatment would seem to be limited to localized alopecia where regrowth may be accelerated, there being little evidence to suggest that the natural course of extensive cases is in any way altered. This disappointing situation seems not to be significantly different from that reported by Kalkoff & Macher (1958) after the use of hydrocortisone.

ACKNOWLEDGMENTS

We wish to thank our colleagues for referring patients to us, and the staff of the Department of Medical Illustration.

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Limitations of paired comparisons of topical drugs

RICHARD R.MARPLES AND ALBERT M.KLIGMAN

Department of Dermatology, University of Pennsylvania,
3400 Spruce Street, Philadelphia, Pennsylvania, U.S.A.

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SUMMARY

The spread of a drug from the site of application to other areas of skin may give rise to false interpretations in paired comparison tests of efficacy. In infected dermatoses treated with topical neomycin the placebo treated site improved clinically and there was almost as great a reduction in the *Staphylococcus aureus* population. Applied to one axilla, topical penicillin engendered resistance among cocci in both axillae. Similarly, washing one axilla with a bacteriostatic soap greatly reduced the microflora in the opposite axilla. When potent antibacterial agents such as chloramphenicol or sodium pyridinethione were applied to a small area of forearm skin, there was sufficient translocation to the opposite side to prevent the great increase in the resident organisms which normally follows wrapping with impermeable plastic film.

For highly potent substances, spread to distant untreated areas has to be considered as a source of error in therapeutic trials.

The application of topical medicaments to equivalently diseased skin on opposite sides of the body provides a simple method of comparing treatments. Two active agents can be compared to determine relative superiority or, simpler still, activity of an agent can be demonstrated against a placebo. The method has become deservedly popular, for the patient serves as his own control. The procedure lends itself to open-ended studies using the principle of sequential analysis in which one merely has to ascertain whether $A = B$, $A > B$, or $A < B$ (Armitage, 1960).

The rules are simple. The lesions should be symmetrical and similar in every way, i.e. of the same severity and quality. The paired comparison method has proved particularly valuable in comparing the effectiveness of topical corticosteroids in chronic dermatoses, e.g. psoriasis, atopic dermatitis, etc. (Stahle, 1967; Dumas & Scholtz, 1972). However, absolute faith in the reliability of any test can lead the unsuspecting into error. The object of this essay is to provide illustrations of fallacious results which come about as a result of translocation of the active material from treated to untreated sites. One may thereby obtain therapeutic effects in untreated areas which are putatively serving as controls. We are here concerned with the treatment of small areas of skin and not extensive surfaces in which absorption could have significant 'distant' effects. The examples we shall present have arisen chiefly from studies of topical antimicrobial agents which strikingly influenced the microflora at sites quite remote from the locale of application.

NEOMYCIN CREAM (1%) VERSUS PLACEBO IN CHRONIC DERMATOSES

A double blind study was conducted in twenty-four patients with chronic dermatoses including eleven in whom lesions were colonized by *Staphylococcus aureus*. The underlying diseases were atopic dermatitis, psoriasis and infected eczematous conditions. Each patient received two coded medications to be applied to symmetrical lesions once daily for 7 days. The patient was shown how to apply the materials so as to avoid cross-contamination. Each was told that two treatments were being studied to determine which was most suitable for the individual and encouragement was given to ensure proper application. The number of aerobic organisms and of *Staph. aureus*/cm^2 of diseased skin was determined before and after treatment by the quantitative detergent-scrub technique (Williamson & Kligman, 1965).

Unexpectedly, especially in those harbouring large numbers of *Staph. aureus*, clinical improvement of an appreciable degree was noted on both sides. The lesions of course did not disappear but were clearly less angry and inflammatory. Indeed, several areas that received no application whatever improved. After the code was broken, it was evident that improvement was greater on the neomycin sites, but the clinical benefit in other diseased areas was nonetheless clearly discernible.

TABLE 1. 1% neomycin cream versus placebo (density of *Staph. aureus*/cm^2)

Neomycin		Placebo	
Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
852,000	21	947,000	8
458	0	144	0
8,170,000	3,650	3,050,000	0
1,200	105	408,000	0
4,470	842	3,420	66
92	0	211	0
2,470,000	0	10,800,000	66
83,700	0	47,000	0
5,260	0	11,000	4,210,000
431,000	84,200	1,750,000	421,000
94,700	0	1,180,000	189,000

The microbiological results provided an explanation of the clinical findings. Firstly, the total number of aerobic organisms fell drastically, from a mean of 11,900 to 405 organisms/cm^2 in the neomycin sites and from 12,900 to 2275 for the placebo. Though the percentage reduction was greater with neomycin, a decrease of more than 80% at placebo sites is an impressive change. Secondly, neomycin completely eliminated carriage of *Staph. aureus* in six of eleven patients and another two showed a reduction of more than 99%. (Table 1). In this respect, effectiveness of the placebo was almost as great, five sites showing complete eradication of the pathogen and two a 99% reduction. In only one placebo site was more *Staph. aureus* recovered after than before treatment.

These results might be explained in two ways. Either the placebo was antibacterial or neomycin had spread over the surface from the site of application. The first possibility was ruled out when a further six patients whose lesions were heavily colonized with *Staph. aureus* were treated with only the placebo cream for 7 days and then subsequently with neomycin alone for a further 7 days (Table 2).

TABLE 2. Density of *Staph aureus*/cm^2 on atopic skin after successive 1 week periods of treatment first with placebo then 1% neomycin cream

	Pre-treatment	Placebo	1% neomycin
	763,000	842,000	0
	253,000	1,890,000	0
	201,000	132,000	126
	1,240,000	842,000	0
	3,160,000	2,680,000	0
	211,000	66,000	132
$\bar{X}$	970,000	1,080,000	43

The placebo did not lower the bacterial population while subsequent application of neomycin drastically reduced the counts.

EFFECT OF 1% TOPICAL PENICILLIN G ON AXILLARY FLORA

Because of warmth and moisture the axilla supports a huge number of resident organisms usually exceeding a million/cm^2. We found previously that the most sensitive indicator of the arrival of an orally administered antibiotic at the skin surface was an increase in the proportion of cocci resistant to the antibiotic (Marples & Williamson, 1969; Marples & Kligman, 1971). This change in the resistance pattern occurred without a decrease in the total number of organisms. For example, when demeclocycline was administered for 3 weeks, coccal resistance rose from 10 to 90% within the first week. With clindamycin coccal resistance rose from undetectable levels to 80% within a 3 week period. In these instances, the initially susceptible organisms were killed by the low surface levels of the antibiotic enabling resistant cells to multiply till the original population was restored.

Five healthy volunteers received 1 ml of 1% aqueous penicillin G to one axilla once daily for 2 weeks. The opposite axilla was not treated. Samples were taken from both axillae before, and after 1 and 2 weeks' of treatment. Resistance was determined by the disc method.

There was no change in the total number of organisms in either axilla. With regard to coccal resistance, however, striking alterations occurred in both (Table 3). By the second week of treatment nearly all of the cocci were resistant to penicillin in both axillae. It seemed very likely that small amounts of

TABLE 3. Effect of topical penicillin on percentage of resistant cocci

	Untreated control		Penicillin	
Subject	Pre-treatment	2 weeks	Pre-treatment	2 weeks
1	15	90	15	100
2	75	100	75	100
3	85	100	85	100
4	5	70	5	*
5	5	100	5	100

* Too few cocci recovered for testing.

penicillin had migrated to the untreated axilla inducing therein a resistant population. Mechanical transfer of resistant cells from the treated axilla is ecologically an unlikely explanation of the findings since in the absence of the drug the tendency is for the population to lose resistance (Marples & Williamson, 1969).

Assay of bacteriostatic soaps in the axilla

The reduction of bacteria in the human axilla has been employed to determine the activity of bacteriostatic soaps (Kooistra, Berman & Carter, 1966). We did two studies of this kind. (1) A 2% hexachlorophene soap was compared to an ordinary soap and (2) the 2% hexachlorophene soap was evaluated against a potent bacteriostatic soap containing tribromosalicylanilide and halogenated carbanilides. Opposite axillae were lathered and rinsed twice daily for 10 days. Microbial samples were obtained before treatment and on the morning after the last washing.

It may be seen from Table 4 that the initial counts were comparable. In the first study, the hexachlorophene soap reduced the bacterial population by 72% in comparison to only 19% on the control side, a significant difference. This result is straight forward and non-provocative. However, the same hexachlorophene soap behaved quite differently in the second study. This time there was a reduction of 96% which was not significantly different from the opposite side treated with a potent bacteriostatic soap.

TABLE 4. Comparisons of the antibacterial effects of soaps in opposite axillae

	First study (forty-one subjects)		Second study (forty subjects)	
	2% hexachlorophene	Non-medicated	2% hexachlorophene	TBS + carbanilides
Pre-treatment	1,518,000*	1,840,000	1,404,000	1,419,000
Post-treatment	424,000	1,484,000	61,500	39,900
% reduction	72	19	96	97
Reduction from original level	s†	ns	s	s
Comparison of reduction	s		ns	

* Total aerobes/cm² (geometric mean).
† Significant, $P<0{\cdot}01$.

Our explanation is that the hexachlorophene soap was only moderately active and did not appreciably affect the organisms in the opposite axilla treated with a non-medicated soap. In the second study, however, translocation from the axilla treated with a potent antimicrobial soap effected a virtual obliteration of the flora on the opposite axilla, conferring a specious potency on the hexachlorophene soap. We have encountered this phenomenon several times. After 10 days of use of a potent bacteriostatic agent in one axilla, a comparable reduction in the bacterial count occurs in the opposite one whether it is untreated or washed with a non-medicated soap.

SPREAD OF ANTIMICROBIAL AGENTS FROM PATCH SITES

These studies were concerned with estimating the length of time an antimicrobial agent could continue to have a repressive effect on the microbial flora at the site of application. The design was to apply a thin film of a 5% concentration of the test agent in hydrophilic ointment USP to a 5 cm square on the volar forearm. The area was immediately covered with a 5 cm square of polyethylene film and sealed with impermeable plastic tape for 24 h. The site was rinsed with water after removal of the dressing. The subjects were then permitted to wash as they pleased for the next 3 days. On the fourth day the treated site and a control site on the opposite forearm were covered with polyethylene occlusive dressing for 24 h, a procedure which hydrates the skin and induces a multiplication of resident organisms to densities in excess of $10^6/cm^2$ (Marples, 1965).

We soon discovered that some agents were inhibiting multiplication of the resident flora on the untreated side. The two most impressive examples were obtained with chloramphenicol and sodium pyridine-thione. As shown in Table 5, the organisms in the untreated sides failed to reach the expected levels, being less than 10^4 in five of eight sites. By contrast, the counts exceeded $1 \times 10^6/cm^2$ in all but one of the controls with erythromycin and trichloro 2-hydroxy-diphenyl ether (Irgasan CH3565—Geigy Chemical). An idea of the amount transferred can be gained by determining the least quantity of chloramphenicol required to prevent multiplication under occlusion. Serial, ten-fold dilutions of this antibiotic were applied to the skin and immediately covered with an occlusive dressing for 24 h. It was found that as little as 4 $\mu g/cm^2$ kept the microflora from increasing appreciably.

TABLE 5. Translocation from patch tests
(bacterial density after 24 h occlusion)

	Treated	Untreated
Chloramphenicol	340	6,800
	30	320,000
	60	3,600
	30	80
Sodium pyridine-thione	10	2,080,000
	10	192,000
	2,400	60
	32,000	2,400
Erythromycin	8,800	3,200,000
	960,000	1,320,000
	5,200,000	64,000,000
	36,000	720,000
Irgasan CH3565	7,600,000	8,800,000
	50	4,270,000
	35,200,000	15,600,000
	260,000	2,720,000
	3,240,000	1,880,000

DISCUSSION

These studies show that translocation of topically applied drugs can sometimes lead to difficulty in the interpretation of paired comparison tests.

The translocation phenomenon can in fact be demonstrated in a very elementary experiment which we have found useful in teaching medical students. A solution of 10% sodium fluorescein is dabbed onto one arm and allowed to dry. A day or two later the students examine the whole body under

Wood's light. The fluorescent dye will be detected in odd patterns at sites far removed from the site of application. Another example is open patch testing with a chemical to which the subject is highly contact allergic. The physician will occasionally be greatly embarrassed when itching eczematous patches occur in sites far removed from the patch.

Whether translocation will compromise a paired comparison test is dependent on the intrinsic activity of the drug. Problems arise only when the agent is so powerful as to exert its effect in microgram quantities. This is not the case with the great majority of topical drugs and chemicals. Highly insoluble substances are also less likely to be translocated in effective quantities. Thus, the paired comparison method has been satisfactorily utilized in assaying topical corticosteroids (Dumas & Scholtz, 1972).

The sceptic could argue that our findings are due to absorption and reappearance at the surface in remote sites. Since normal skin possesses a very effective barrier and considering the great dilution by body fluids this mechanism is hardly conceivable. With penicillin, a distant effect via absorption cannot be considered at all for we found that even high oral doses produced barely detectable effects on the microflora (Marples & Kligman, 1971). Washing and bathing is probably the chief means by which topically applied substances spread over the surface. Other agencies include transfer on clothing, by body movements, and sweating. There may be unperceived 'currents' and streams on the skin as in the oceans (Lewis *et al.*, 1969).

Though the examples presented here relate to antimicrobial agents, other illustrations come readily to mind. It has been noted that repeated topical application of potent carcinogens can lead to neoplasia at sites other than the area of application. We encountered still another example when appraising the effectiveness of retinoic acid in acne vulgaris. When the medication was applied to one side of the face we found clear cut evidence of increased epidermal proliferation by thymidine radioautography on the opposite side even though there was no visible reaction. In their half-face study McGillis *et al.* (1971) observed peeling and erythema on the untreated side of some patients. Spread to a contiguous area is of course easier to understand. Some experimenters have tried to use half-scalp applications in assessing anti-dandruff agents. In our experience the translocation phenomenon utterly nullifies such a tactic. We applied 1% aqueous sodium pyridine-thione to one-quarter of scalps uniformly involved with severe dandruff; invariably the entire scalp cleared after 10 days of daily applications.

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A method for the assay of topical corticosteroids*

R.MARKS, D.PONGSEHIRUN AND T.SAYLAN

Department of Pathology, Institute of Dermatology, Lisle Street, Leicester Square, London, W.C.2

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SUMMARY

The dorsal skin of the hairless mouse when 'stripped' with sticky tape proved to be a suitable model for a study of the antimitotic effect of betamethasone-17-valerate and hydrocortisone. The application of 1% betamethasone-17-valerate and 1% hydrocortisone creams under occlusive dressings for 24 h produced a three- to four-fold drop in the colcemid-induced mitotic index as compared to a control application.

The antimitotic effect of the betamethasone-17-valerate preparation started at or before 5 h and continued for at least 4 days after removal of the active material. It was shown in addition, that 1% betamethasone-17-valerate also reduced the number of cells entering DNA synthesis. The antimitotic effect of hydrocortisone was detected at a concentration of 10^{-2} and that of betamethasone-17-valerate was detected at a concentration of 10^{-4}. It is suggested that the animal model used is a useful one for assaying the potency of topical corticosteroid compounds.

The most widely used method for the assay of the potency of topical corticosteroid preparations is the McKenzie Stoughton vasoconstrictor test. Although the vasoconstrictor test has the advantages of rapidity and simplicity it has certain drawbacks. In the first place it relies on an action, which, while it perhaps contributes to the therapeutic effect of topical corticosteroids is certainly not their main mode of action. Secondly, it requires human volunteers, and lastly the measured effect is interpreted subjectively. For these reasons there has been a need for another way of assaying topical corticosteroids. In this communication we report our experience with the use of an animal model which relies on the antimitotic effect of corticosteroids for the assay of the potency of topical corticosteroid preparations.

METHOD

Mature male hairless mice of the Harwell strain (hr/hr) were used. The method of Hennings & Elgjo (1970) was employed to artificially boost the mitotic rate of the dorsal skin. Sticky tape was applied five or six times to the dorsal skin of the hairless mice. This was usually sufficient to remove the horny layer and enabled the 'glistening membrane' to be seen. After this manoeuvre, 0·5 cm lengths of the topical preparation under test (all preparations used were in identical tubes with identical nozzles) were squeezed onto the stripped dorsal skin of each mouse. This ensured that each mouse received a similar quantity of topical medicament (0·5 cm of cream weighed 68·2±0·8 mg).

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* Paper read at meeting of the Investigative Group of the British Association of Dermatology, January 1972.

Each mouse then had a dressing applied (cotton gauze lined with a plastic film) which was secured with strapping. The dressing stayed on for periods of from 5 to 196 h. Four hours before sacrifice the mice were injected intraperitoneally (through the dressings) with a stabilized solution of colcemid. The colcemid solution was made up as follows: colcemid—0·1%, 96% ethanol—5·3%, propylene glycol—10%, monosodium phosphate—0·2%, sodium phosphate—0·15%, water to 100%.

Each mouse received 0·15 ml of this solution. In some instances 30 μCi of tritiated thymidine (specific activity 26 Ci/mmol—Radiochemical Centre, Amersham) was added to the colcemid solution and injected with it. The control preparation, which also was the vehicle for the active preparations, contained: cetomacrogol, cetostearyl alcohol, chlorocresol, liquid paraffin, white soft paraffin, phosphate buffers and water.

The corticosteroid preparations tested were:

(a) betamethasone 17-valerate in concentrations of from 1% in ten-fold dilutions down to 0·00001% (10^{-5}) and

(b) hydrocortisone in concentrations of from 1% in ten-fold dilutions down to 0·001% (10^{-3}). Each preparation was tested on a batch of at least six mice.

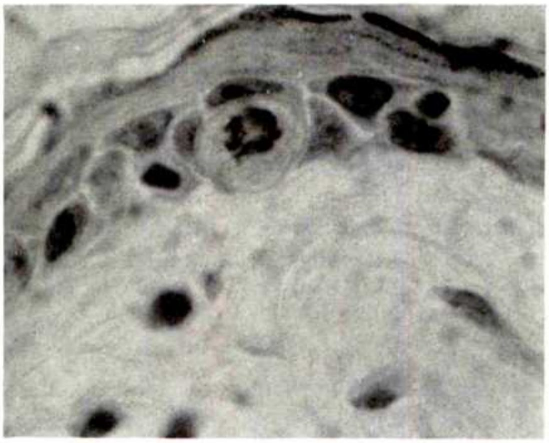

FIGURE 1. Photomicrograph of section of skin from back of hairless mouse treated 4 h previously with colcemid showing basal cell in metaphase.

After sacrifice the dorsal skin to which the preparation had been applied, was removed and fixed in 10% buffered formalin. It was then dehydrated and embedded in paraffin wax and sectioned. Multiple sections were mounted on slides and stained with haematoxylin and eosin.

For the preparation of autoradiographs, the dipping method of Joftes & Warren (1955) was employed. Ilford nuclear emulsion (K2) was used with exposure times of 3 and 4 weeks.

For estimation of the mitotic index cells in the prophase, anaphase and metaphase stages were counted (Fig. 1). Care was taken to exclude degenerate cells which at times could closely resemble mitotic figures. Basal clear cells (melanocytes) could also resemble mitotic figures and these were carefully excluded as well. The number of cells in mitosis occurring in a total of 1500 basal cells, from at least three sections, was counted for each mouse.

To quantitate the labelling index the number of labelled basal and suprabasal cells was estimated as a proportion of the total number of basal cells. A cell was counted as labelled if it possessed more than six autoradiographic grains over it. Between 500 and 1000 basal cells were counted for each specimen. Many of the sections were checked 'blindly' by three other observers.

RESULTS

Stripping itself produced a two-fold rise in mitotic index (see Table 1). The application of the unmedicated cream itself both to 'stripped' and to 'unstripped' skin appeared to have a slight antimitotic effect (see Table 1). This was statistically significant for the 'stripped' skin, $P<0.001$, but only marginally so for the 'unstripped' skin, $P<0.1>0.05$.

Application of both 1% hydrocortisone and 1% betamethasome-17-valerate preparations had an antimitotic effect (see Table 1) both on 'stripped' and 'unstripped' skin when compared with the control preparations. Ten-fold dilutions of both the hydrocortisone and betamethasone-17-valerate preparations were then tested and it was found that a significant antimitotic effect was produced by a concentration of 10^{-4} ($P<0.01$) of betamethasone-17-valerate but not by a concentration of 10^{-5}. Hydrocortisone preparations proved to be significantly antimitotic even at concentrations of 10^{-2} ($P<0.01$) but not at 10^{-3}. It was of interest to note that the antimitotic effect did not appear to be an all-or-none phenomenon.

TABLE 1. The effect on colcemid-induced mitotic index of various applications on stripped or unstripped dorsal skin of hairless mice after 24 h

Application	Stripped/unstripped	Mitotic index (%) Mean	Mitotic index (%) SE mean
None	Unstripped	6·9	±0·14
None	Stripped	12·0	±0·90
Control	Unstripped	5·5	±0·21
Control	Stripped	9·8	±1·10
1% β-17-val	Unstripped	1·6	±0·17
1% β-17-val	Stripped	2·4	±0·16
10^{-1}% β-17-val	Stripped	3·2	±0·22
10^{-2}% β-17-val	Stripped	2·1	±0·14
10^{-3}% β-17-val	Stripped	2·4	±0·30
10^{-4}% β-17-val	Stripped	7·0	±0·08
10^{-5}% β-17-val	Stripped	11·0	±0·90
1% hydrocortisone	Stripped	3·2	±0·22
10^{-1}% hydrocortisone	Stripped	3·8	±0·70
10^{-2}% hydrocortisone	Stripped	5·5	±0·29
10^{-3}% hydrocortisone	Stripped	8·2	±1·10

β-17-val, betamethasone-17-valerate.

The results of the effects of 1% betamethasone-17-valerate cream and the control preparation on the mitotic index at various periods after stripping and applying the preparation are given in Table 2 and set out graphically in Fig. 2. A significant antimitotic effect from the steroid preparation was evident at 5 h after application of the preparations. The maximal antimitotic effect was seen at 18 h and an antimitotic effect was still evident at 120 h after the start of the experiment (96 h after removal of the application). There was no difference between the effects of the preparations observed at 196 h after the start of the experiment.

From Table 3 it can be seen that 1% betamethasone-17-valerate considerably reduced the autoradiographic labelling index also as compared to the control preparation at the two times 18 and 48 h.

TABLE 2. The effects of the control application and 1% betamethasone-17-valerate cream (β-17-val) on the colcemid-induced mitotic index of 'stripped' dorsal skin at increasing time intervals after application (the applications were removed at 24 h)

	Mitotic index means ± SE	
Time after application	Control	1% β-17-val
5	6 6±0·65	4·2±0·67
8	6·8±0·19	2·8±0·21
18	13·6±1·0	2·8±0·09
24	9·8±1·1	2·4±0·16
48	10·9±0·64	3·1±0·45
72	7·3±0·17	4·9±0·30
120	5·7±0·48	3·2±0·16
192	7·3±0·17	7·2±0·15

The differences between the control and active preparations were statistically significant at both times ($P<0·01$).

DISCUSSION

The glucocorticoid hormones have an antimitotic effect on a number of tissues. In particular they have been found to have an antimitotic effect on lymphocytes (Stevens, Colessides & Dougherty 1966), on fibroblasts (Berliner & Ruhmann, 1966) and on some cell lines in culture (Kline *et al.*, 1957).

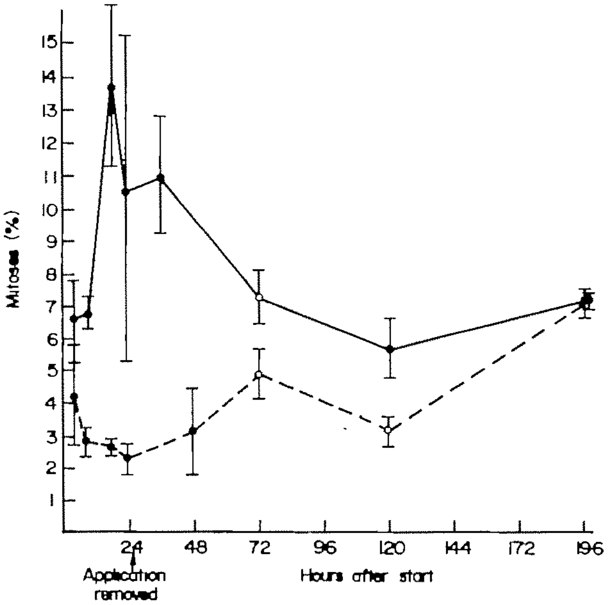

FIGURE 2. Mean mitotic indices of dorsal skin of groups of mice treated either with 1% betamethasone-17-valerate cream (broken line) or the control preparation (unbroken line) at increasing time intervals, after applying the test materials. The SE of the mean for each mean is also indicated.

There is also good evidence from previous work to suggest that the corticosteroids have a similar antimitotic action on the epidermis. Thus, Reaven & Cox (1968) found that hydrocortisone reduced the number of cells entering DNA synthesis in adult human skin in culture and Marks *et al.* (1971) found a reduced rate of thymidine incorporation in adult human skin that had been treated with triamcinolone acetonide. In addition, Chaudhury, Halberg & Bittner (1971) detected a reduced mitotic rate in the epidermis of mice treated with hydrocortisone or 9-alpha-fluorocortisone and Hennings & Elgjo (1971) detected a 60% decrease in mitotic rate in the stripped dorsal epidermis of mice after treatment with hydrocortisone.

It was of interest to find that the antimitotic effect lasted for at least 5 days after application (4 days after removal of the dressings) although the mechanism of this prolonged effect is entirely unknown. As the areas to which the cream was applied had been stripped of horny layer it is presumed that a reservoir effect of the stratum corneum could not have been responsible. The reduction in the number of cells entering the DNA synthesis phase within epidermis treated by the corticosteroids suggests that the effect of the corticosteroids may be at an early stage of the mitotic cycle and the mechanism of action of these compounds on the epidermis is now being studied in detail (Marks *et al.*, unpublished data).

TABLE 3. Labelling indices 18 and 48 h after application of 1% betamethasone-17-valerate (β-17-val) and control creams. The antimitotic effect is statistically significant at both times

Time after application (h)	Application	Mean labelling index	SE mean	Significance
18	1% β-17-val	10·4	±2·0	$P = <0·01$
	Control	35·5	±4·0	
48	1% β-17-val	14·8	±4·2	$P = <0·01$
	Control	30·2	±3·7	

Fisher & Maibach (1971) suggested that the reduction in mitotic rate produced by corticosteroids might be used as an assay system. We have shown in this investigation that it is indeed possible to use the antimitotic effect to assay the potency of topical corticosteroids and we believe that an assay conducted in this way might have more relevance to the clinical uses of these compounds than the methods now employed. It is possible that some of the antimitotic effect observed might well be due to systemic absorption of the corticosteroid, but whether this is so or not does not affect the validity of the assay system.

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Induction by topical corticosteroids of skin enzymes metabolizing carcinogenic hydrocarbons

M.H.BRIGGS AND MAXINE BRIGGS

Department of Biochemistry, University of Zambia, P.O. Box 2379, Lusaka, Zambia

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SUMMARY

Epidermal aryl hydrocarbon hydroxylase activity of homogenates from mice treated for 7 days with various topical corticosteroid ointments was significantly higher than in preparations from animals receiving steroid-free ointment bases, or no treatment. As determined by 3,4-benzopyrene oxidation, enzyme activity was also greatly increased 24 h after small doses of various polycyclic hydrocarbons given by the intraperitoneal route. Inhibition of enzyme activity was observed in skin homogenates from both control and treated animals following addition *in vitro* of 7,8-benzoflavone, but not with dimethylsulphoxide.

Topical corticosteroid preparations are one of the most widely used pharmaceuticals in dermatological practice, but little is known of their effects on epidermal cell biochemistry. Steroid hormones of all types are well known for their ability to induce marked changes in enzymes of many tissues. Some of these changes are in activity of microsomal oxidases, for which steroids themselves can serve as substrates. Microsomal oxidases have low substrate specificity and are important in the metabolism of numerous foreign compounds (Parke, 1968).

Large increases in liver microsomal oxidation of aromatic hydrocarbons occur in rat following oral administration of dexamethasone acetate (9α-fluoro-16α-methyl-11β,17α,21-tri-hydroxy-1,4-pregnadiene-3,20-dione 21-acetate), betamethasone acetate (9α-fluoro-16β-methyl-11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione 21-acetate), ethylestranol (17α-ethyl-4-estren-17β-ol), or spironolactone (7α-thioacetyl-17β-hydroxy-4-androsten-3-one 17α-propionic acid-γ-lactone) (Somogyi, *et al.*, 1971). Hydrocarbon oxidation by microsomes is catalysed by enzyme systems known as 'aryl hydrocarbon hydroxylases' (AHH), which require a haemoprotein related to cytochrome P-450, together with reduced adenine-nicotinamide-dinucleotide phosphate (NADPH) and divalent cations (Venkatesan, Arcos & Argus, 1971).

Many polycyclic hydrocarbons are oxidized by microsomal AHH, including 3,4-benzopyrene (benzo(a)pyrene), 1,2-benzanthracene (benz(a)anthracene), 9,10-dimethyl-1,2-benzanthracene (7,12-dimethylbenz(a)anthracene), 3-methylcholanthrene, 1,2,5,6-dibenzanthracene (dibenz(a,h)anthracene and anthanthrene (dibenzo(c,d–j,k)pyrene) (Conney & Burns, 1964). Low levels of microsomal AHH are present in mammalian epidermis, but are greatly increased by hydrocarbons applied topically or by intraperitoneal administration (Gelboin, Weibel & Diamond, 1970).

Correspondence: Professor Michael Briggs, Maple Cottage, Little Eaton, Derby.

While AHH has generally been considered as a detoxication mechanism for hydrocarbons (Parke, 1968), there is now evidence that the enzyme system is involved in conversion of some hydrocarbons to 'proximate carcinogens', i.e. metabolites which initiate cellular changes leading finally to cancer (Gelboin, Huberman & Sachs, 1969). As skin AHH is induced by topically applied hydrocarbons, while the liver enzyme is known to be induced by oral corticosteroids, we decided to investigate the effect of topical corticosteroids on microsomal AHH activity of epidermis.

METHODS AND MATERIALS

Animals

All studies were conducted on groups of locally-bred Swiss-strain albino mice. Male animals weighing 20–25 g were used exclusively. They were caged in groups of four and given free access to water and a commercially available laboratory animal food in pellet form. Hair was removed from the animals' backs by close shaving. Animals with damaged skin were not used.

Pharmaceuticals and chemicals

Aromatic hydrocarbons were purchased from commercial suppliers, but were re-crystallized before use and physical properties compared with standard values. All solvents were re-distilled, while buffer solutions and reagents for enzyme assay were prepared fresh on the day of use. Purity of crystalline corticosteroids was checked by thin-layer chromatography. Topical corticosteroid preparations were commercially available ointments with the following composition: 'Betnovate' (Glaxo) 0·1% betamethasone valerate (9α-fluoro-16β-methyl-$11\beta,17\alpha,21$-trihydroxy-1,4-pregnadiene-3,20-dione 17α-*n*-valerate), 'Propaderm' (Allen and Hanbury) 0·025% beclomethasone dipropionate (9α-chloro-16β-methyl-$11\beta,17\alpha,21$-trihydroxy-1,4-pregnadiene-3,20-dione $17\alpha,21$-dipropionate),'Synalar' (Imperial Chemical Industries) 0·025% fluocinolone acetonide ($6\alpha,9\alpha$-difluoro-$11\beta,16\alpha,17\alpha,21$-tetrahydroxy-1,4-pregnadiene-3,20-dione 16,17-acetonide), 'Ultradil' (Schering Chemicals) 0·1% fluocortolone (6α-fluoro-16α-methyl-$11\beta,21$-dihydroxy-1,4-pregnadiene-3,20-dione) plus 0·1% fluocortolone 21-caproate, 'Ultralanum Plain' 0·25% fluocortolone plus 0·25% fluocortolone 21-caproate.

Enzyme assay

Mice were killed by cervical dislocation and skin of treated areas removed immediately with scissors and placed in cold 0·25 M sucrose–0·05 M tris (tris-hydroxymethyl-aminomethane) buffer pH 7·5. Epidermis was removed by scraping with a scalpel, then homogenized in a Potter–Elvehjem type instrument with a small amount of buffer. AHH activity was determined by incubating 0·1 ml homogenate with 0·36 μmol NADPH, 3·0 μmol $MgCl_2$ and 80 mμmol/ml 3,4-benzopyrene (dissolved in methanol) in a total volume of 1·0 ml made up with buffer. After 30 min at 37°C, the reaction was stopped by adding 1·0 ml ice-cold acetone, and the mixture extracted with 3·25 ml *n*-hexane with shaking for 10 min. Organic phase was removed and 1·0 ml re-extracted with 3·0 ml 1 N NaOH solution. Concentration of 8-hydroxy-3,4-benzopyrene in the extract was determined with an Eppendorf fluorimeter by activation at 390 nm and measurement at 520 nm, then comparison with a standard curve prepared from authentic compound.

Protein concentration of homogenates was determined by the method of Lowry *et al.* (1951) and AHH activity calculated as pmol (10^{-12}M) 8-hydroxy-3,4-benzopyrene formed/mg protein/h under the conditions of the assay.

Using pure compounds, each corticosteroid was tested separately to determine its effects on AHH activity when added *in vitro* at 10^{-4}M.

Experiments

(1) A comparison was made of various hydrocarbons for their induction effects on skin AHH when administered by the intraperitoneal route. Each animal of the test groups received 3·0 mg hydrocarbon dissolved in arachis oil, while controls received only oil. Animals were killed 24 h after the injection.

(2) Topical corticosteroids were tested for enzyme induction effects by applying 0·5 g ointment twice daily to shaved backs of test animals. A control group was untreated, while two further groups received twice daily applications of 0·5 g ointment bases (steroid-free). Animals were killed at the end of the seventh day of ointment application.

(3) Some groups received topical corticosteroid treatment for 7 days, as well as 3·0 mg 1,2-benzanthracene i.p. on the final day, 24 h before being killed.

(4) Particular homogenates from individual animals were assayed for AHH activity before and after addition of 10^{-6}M 7,8-benzoflavone or 0·9 M dimethylsulphoxide (DMSO).

(5) Three groups, each of twenty mice, received 0·5 g ointment twice daily. One group received steroid-free ointment base, a second 'Synalar', while the third received 'Ultralanum Plain.' After 7 days, ointment treatment was stopped and all animals received 10 μg 9,10-dimethyl-1,2-benzanthracene in 0·2 ml acetone once weekly for 10 weeks. Animals were examined for skin cancers at 20 weeks.

Statistics

Mean values for enzyme activity of skin homogenates were calculated for each group of treated mice and standard deviations were derived. Differences were evaluated for statistical significance by Student's *t* test and calculation of *P* values.

TABLE 1. Induction of epidermal AHH activity by hydrocarbons

Hydrocarbon administered*	AHH activity (pmol/mg protein/h) (mean ± SD)	Mean increase over controls (units)	Enzyme increase/μmol hydrocarbon (units)
None (controls: oil only)	79 ± 19	—	—
3,4-Benzopyrene	1015 ± 60	936	78
1,2-Benzanthracene	1039 ± 72	960	77
3-Methylcholanthrene	488 ± 86	409	37
9,10-Dimethyl-1,2-benzanthracene	1178 ± 68	1099	92

* 3·0 mg i.p. in arachis oil 24 h previously.

RESULTS

The effects of four different aromatic hydrocarbons on induction of epidermal AHH activity are shown in Table 1. The increase in treated groups over the controls is statistically significant ($P < 0{\cdot}001$) in all cases, though the greatest increase is seen in animals given 9,10-dimethyl-1,2-benzanthracene. Recalculation of enzyme increases in terms of activity per μmol hydrocarbon has little effect on the results, for all four compounds have similar molecular weights.

Table 2 summarizes effects of various topical corticosteroid ointments on epidermal AHH activity. Neither of the two steroid-free ointment bases had any significant effect on the enzyme, but four of

TABLE 2. Effect of topical corticosteroids on epidermal AHH activity

Treatment*	AHH activity (pmol/mg protein/h) (mean ± SD)	Mean increase over controls (units)	Enzyme increase/μmol steroid (units)
None (controls)	77 ± 15	—	—
Ointment base A	78 ± 11	—	—
Ointment base B	82 ± 20	—	—
Betnovate	450 ± 56	373	183
Propaderm	376 ± 38	299	650
Synalar	85 ± 21	8	15
Ultradil	442 ± 58	365	76
Ultralanum Plain	788 ± 63	711	61

* 0·5 g ointment applied twice daily for 7 days.

five active ointments induced large increases which are statistically significant ($P < 0{\cdot}001$) in all cases. The change with 'Synalar' does not vary significantly from enzyme activity seen in animals treated with ointment bases.

Pure corticosteroids added *in vitro* at 10^{-4}M to homogenates had no effect on AHH activity.

As concentration of corticosteroids in the five ointments varies considerably (0·025–0·25%), while molecular weights of the active components range from 377 to 550, increases in AHH activity were re-calculated to take account of molar concentrations of corticosteroids in ointments (column 4 of Table 2). It will be seen that individual corticosteroids vary markedly in their induction capacities for AHH, so that the increase seen with any particular ointment is determined by both the concentration of steroid in the ointment and the enzyme induction capacity of that steroid.

Table 3 shows the effects of combined treatment with topical corticosteroid and i.p. hydrocarbon. Increase in epidermal AHH activity following 1,2-benzanthracene and 'Ultralanum Plain', or 'Synalar', was not significantly different to the increase induced by 1,2-benzanthracene alone.

Results of investigations on effects of 7,8-benzoflavone or DMSO on AHH activity in homo-

TABLE 3. Effect of combined topical corticosteroid and parenteral hydrocarbon on epidermal AHH activity

Topical corticosteroid*	Hydrocarbon†	AHH activity (pmol/mg protein/h) (mean ± SD)
None	None	77 ± 15
None	1,2-benzanthracene	1039 ± 72
Ultralanum Plain	None	788 ± 63
Ultralanum Plain	1,2-benzanthracene	995 ± 58
Synalar	None	85 ± 21
Synalar	1,2-benzanthracene	985 ± 63

* 0·5 g ointment applied twice daily for 7 days.
† 3·0 mg i.p. in oil on day 7, 24 h before enzyme assay.

TABLE 4. Effect of 7,8-benzoflavone or DMSO on epidermal AHH activity

Treatment group (individual skin homogenate)	AHH activity (pmol/mg protein/h) Additions: None (100%)	7,8-benzoflavone (10^{-6}M)	DMSO (0·9 M)
None	84	63 (75%)	82 (98%)
9,10-dimethyl-1,2-benzanthracene	1192	802 (68%)	1184 (99%)
3-methylcholanthrene	568	335 (59%)	558 (98%)
Ultralanum Plain	825	578 (70%)	805 (97%)
Synalar	92	65 (71%)	90 (98%)

genates from treated and untreated animals are given in Table 4. In all cases, 7,8-benzoflavone reduced enzyme activity significantly, while DMSO had no effect.

It will be seen from Table 5 that skin tumours were found in five of eighteen survivors pre-treated with steroid-free ointment base, then exposed to 9,10-dimethyl-1,2-benzanthracene. Tumour incidence in mice pre-treated with 'Synalar' was similar (six of nineteen), but fifteen of sixteen survivors pre-treated with 'Ultralanum Plain' had skin cancer. This difference is statistically significant ($P<0{\cdot}01$).

TABLE 5. Induction of skin cancer in mice treated by topical corticosteroids

Pre-treatment*	Treatment†	No. of mice: Starting	At 20 weeks	Skin tumours: Animals with tumours	Total tumours	Tumours per mouse
Ointment vehicle	10 μg DMBA	20	18	5	52	10·4
Ultralanum Plain	10 μg DMBA	20	16	15	171	11·4
Synalar	10 μg DMBA	20	19	6	59	9·8

* 0·5 g ointment applied daily for 7 days.

† 10 μg 9,10-dimethyl-1,2-benzanthracene (DMBA) in 0·2 ml acetone applied once a week for 10 weeks.

DISCUSSION

Results presented above indicate that epidermis of untreated mice possesses low level AHH activity, but treatment by either parenteral hydrocarbons or topical corticosteroids induces statistically significant increases in most cases. Evidence from studies of AHH induction in other tissues (Venkatesan *et al.*, 1971) suggests that the mechanism involves *de novo* synthesis of enzyme protein molecules, perhaps by de-repression of messenger-RNA biosynthesis in the nucleus.

It is uncertain whether synthetic corticosteroids can serve as substrates for AHH, but many other steroids are oxidized by microsomal enzyme systems sharing features in common with AHH. Increased steroid hormone oxidation has been demonstrated by Orrenius, Das & Gnosspelius (1969) in hepatic microsomes from animals pre-treated with aromatic hydrocarbons.

Several different AHH systems are known (Wiebel *et al.*, 1971) and may be distinguished by sensitivity to inhibitors. Hepatic AHH from untreated rats is inhibited by DMSO, but not by benzoflavones. In contrast, AHH in lung, kidney and skin of untreated animals is inhibited by benzoflavones, but not by DMSO. Hydrocarbon-induced AHH in lung, kidney or skin remains sensitive to benzoflavones, but induced enzyme in liver is not inhibited by either benzoflavones or DMSO. Surprisingly, hepatic AHH induced by phenobarbitone is inhibited by both benzoflavones and DMSO.

Further evidence of differences in AHH comes from studies of adrenal necrosis induced in rats by 9,10-dimethyl-1,2-benz-anthracene (Somogyi *et al.*, 1971). Untreated animals metabolize this hydrocarbon to a 10-hydroxymethyl metabolite, which is a potent adrenal necrotic agent. Following treatment by oral corticosteroids, 9,10-dimethyl-1,2-benzanthracene no longer induces adrenal necrosis, for hepatic AHH induced by the steroids metabolises the hydrocarbon to ring-oxygenated compounds, rather than the 10-hydroxymethyl compound.

Considerable evidence has accumulated (Gelboin *et al.*, 1969) that ring-hydroxylation of hydrocarbons is associated with formation of 'proximate carcinogens'. While the ring-hydroxylated hydrocarbons are probably not themselves carcinogens, an intermediate such as an epoxide may well be. The mechanism of carcinogenesis by aromatic hydrocarbons involves formation of covalent bonds between the hydrocarbon and DNA molecules, and these bonds require the oxygen atom introduced by AHH. Simultaneous administration of benzoflavones (to inhibit AHH) with a carcinogenic hydrocarbon often prevents tumour formation, while benzoflavones given a few hours after the hydrocarbon have no protective effect.

Our results suggest that mouse skin AHH induced by topical corticosteroids is the same system as that induced by hydrocarbons, which is known to be pro-carcinogenic (Gelboin *et al.*, 1970). It seems likely that a similar effect may occur in skins of human patients treated by topical corticosteroids. Microsomal enzyme systems of low substrate specificity are widely distributed throughout the animal kingdom. Induction by steroid hormones of microsomal oxidases acting on hydrocarbons has even been demonstrated in an invertebrate, the housefly (Yu & Terriere, 1971).

In the absence of evidence to the contrary, it seems wise to assume that increases occur in epidermal AHH activity of humans receiving topical corticosteroid therapy. As the enzyme seems intimately involved in hydrocarbon carcinogenesis, such patients may be at increased risk of skin cancer from hydrocarbons with which they come in contact. Low concentrations of potentially carcinogenic hydrocarbons are present throughout the human environment, including air, water and foods, but are present in much larger amounts in many petroleum products. Patients whose work brings them into contact with hydrocarbons, for example, workers in the oil industry, motor trade, gas works, etc., may be a special risk for topical corticosteroids, which should not be prescribed, unless essential. In those cases where they are essential, a product should be selected with the lowest AHH induction capacity.

Horton, Dreal & Bingham (1966) have pointed out that corticosteroids may be pro-carcinogenic in skin cancer through a second mechanism unrelated to AHH induction: suppression of immunological defense mechanisms of the epidermis.

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A clinical trial with hydrocortisone butyrate cream in eczema

M.K.POLANO AND P.KANAAR

Department of Dermatology, University Hospital, Leiden, The Netherlands

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SUMMARY

In a double blind trial 0·1% hydrocortisone butyrate cream gave after a week a significantly better therapeutic response in eczema than 0·1% triamcinolone acetonide ($P<0·05$) and hydrocortisone acetate. The methods for clinical trials are discussed.

In a previous paper (Polano *et al.*, 1970) the results of a clinical trial were discussed, comparing 0·1% hydrocortisone butyrate (HB)* with a 0·1% triamcinolone acetonide (TA) and 1% hydrocortisone acetate (HA), all three in an o/w cream applied under plastic occlusion, in patients with psoriasis. Now we are able to report on a trial comparing these three corticosteroids applied without occlusion, in eczema.

METHOD

Because patients with symmetrical eczema lesions are rare in our in-patient department, we decided not to use the method of paired comparisons but to treat alternate patients with one of the three drugs.

We admitted to the trial out-patients suffering from neurodermatitis, seborrhoeic eczema, or unspecified eczema (eczema vulgare) deliberately excluding patients with allergic contact dermatitis. Patients who had been treated with potent corticosteroids in the last month before the trial were also excluded.

The patients were provided with a supply of cream for 2 weeks, in tubes containing 0·1% HB or 0·1% TA or 1% HA cream, labelled with randomized code numbers.

The condition of the patients was evaluated at the beginning of the trial, after a week, and after 2 weeks. Score points ranging from 5 to 0 were given separately for itching, lichenification, infiltration, erythema, exudation, crusts, scales, vesicles and papules, a score of 5 indicating severe involvement, and 0 absence of the symptom or sign.

RESULTS

After decoding the score sheets, four patients proved not to have completed this schedule, one of them in the HB group, one in the TA group, and two in the HA group. The results in the patients who completed the trial are shown in Fig. 1 and Table 1.

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* Hydrocortisone butyrate was developed by the R.N.F.I. and is marketed in various vehicles as Locoid®.

The data in the Tables indicate that the severity of the condition at the start of the trial was approximately the same in all three groups.

Figure 1 shows that the therapeutic effect varied between the three drugs. After 1 week, all produced improvement, but HB considerably more than HA and TA. After the second week, no further improvement occurred in the HA group, but under TA and HB treatment, further improvement was clearly obtained in the second week.

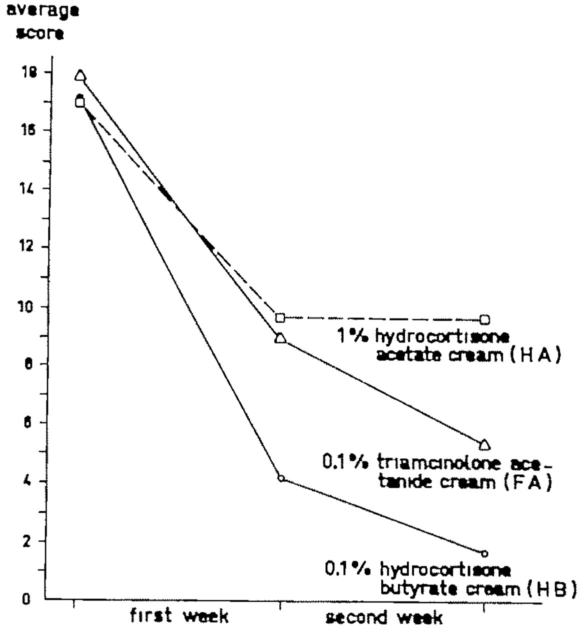

FIGURE 1. Decrease of the score indicates the reduction in the severity of eczema in the three groups of patients.

TABLE 1. Eczema trial. Mean scores of the patients at the beginning of the trial and after 1 and 2 weeks

	HB	TA	HA
Start	17·9	17·9	17·0
1 week	4·2	9	9·7
2 weeks	1·7	5·4	9·7

We felt that the improvement expressed as a percentage of the initial score would be the most relevant indication of the efficacy of the treatment. These percentages are given in Table 2.

Our statistical consultant, Mr H. de Jonge, Senior Lecturer in Medical Statistics, tested the statistical significance of the differences between the percentages for the three steroids.

With the distribution free test of Kruskal and Wallis the difference in effect between the three corticosteroids after 1 and 2 weeks proved to be significant ($P<0{\cdot}01$). Comparison between the individual preparations with Wilcoxon's two sample test showed the difference between HB and TA

TABLE 2. Eczema trial. Scores after 1 and 2 weeks, expressed as percentages of the initial scores, in twenty-six patients.

	HB		TA		HA	
	1 week	2 weeks	1 week	1 week	1 week	2 weeks
	38	15	76	62	71	105
	36	0	50	33	56	89
	21	14	60	64	77	69
	15	0	33	0	85	69
	21	0	15	0	50	70
	20	0	56	28	53	37
	22	20	50	22	21	7
	38	18	65	10	50	18
	12	8	24	14		
Median	21	8	50	22	54·5	69

after 1 week to be significant in favour of HB ($P<0{\cdot}05$). After 2 weeks the difference in scores between HB and TA, although favouring HB, was not statistically significant, while both were still significantly superior to HA.

DISCUSSION

In eczema the 0·1% HB cream gave statistically better therapeutic results than TA and HA after a week. If we compare these results with those obtained in the psoriasis trial we reported in 1970 we see that, in psoriasis under occlusion, no difference was observed between TA and HB, both being equally effective and superior to HA.

The results of these two trials make it clear that statements on the merits of topical drugs should be explicitly limited to the conditions treated in the trial.

ACKNOWLEDGMENT

Dr L. van Wijk of the Clinical Research Department of the R.N.F.I. provided us with the test material.

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Comment

BIOCHEMICAL VARIABILITY IN THE SKIN

In recent years it has been almost a fashion, certainly a habit, for scientists in dermatology to begin reviews of their subject with apologies for the uncultivated state of the field and the poverty of the crops so far yielded (Cotton & Mier, 1972). To some extent such guilt feelings are well justified since there probably remain more diseases of 'unknown etiology' within dermatology than in any other organ-limited field of medicine. But now it seems that the basic problem of the variability of the skin from site to site, depth to depth and time to time which has been our greatest philosophical problem, may prove to be our greatest scientific strength. Hsia (1972) has recently pointed out some of the factors that make skin such a useful organ for the biochemist to study and I should like to extend this by collecting together some recent data concerning the local and temporal variability of the skin and attempt to show its relevance to dermatology in general.

The term variability is used by different authors in different ways. Mali (1964) has described what might be called the physiological variability of the skin, the changes that can be seen to be occurring within, ultimately, an individual cell as a response to its environment. What I should like to draw attention to here are the biochemical differences between cells that occur as a function of time or their different metabolic programming; these include the differences between different cell types, differences in overall biochemistry arising out of the variable concentration of cell types and a third, less obvious, difference arising out of differences between morphologically identical cells (see Davidson, Nitowsky & Childs, 1963). In what follows the term 'variability' will subsume any or all of these possibilities.

LOCAL AND TEMPORAL VARIABILITY

Morphology and function

There is little here that needs labouring since the first impression that one gets of skin is that it indeed varies widely within an individual in terms of both morphology and function. Different regions support and elaborate different adnexa, the skin of the infant develops slowly into the radically different skin of the aged, and diseases wax and wane in their patchy courses. There exists a wealth of data on the variability of form and function in the skin and most of it is too familiar to need reviewing here.

Variability is perhaps the basic characteristic of skin (rather than just an odd phenomenon about which there is any disagreement).

GROSS BIOCHEMICAL VARIABILITY

Considerably less has been reported about local variations in skin biochemistry; in fact the possibility of such variations has often been ignored and many reports describe work using skin with no reference to site or to the age of the subjects. The advent of techniques for microdissection (Lowry, 1953) and enzyme micro-assay (Lowry *et al.*, 1953), their application to skin (Hershey, 1964) and subsequent definitive employment by Adachi and co-workers (Adachi & Yamasawa, 1968) has shown that enzyme activities may vary widely between various adnexa and various epidermal layers. Consequently even on a weight basis (per unit weight of tissue) enzyme activities will vary with the local concentration of such adnexa or the relative development of the various layers. Even taking this into account the determinations of enzyme activities (i.e. V_{max}) is notoriously unreliable in the skin and only abnormalities such as the complete absence of an enzyme will be revealed by activity measurements. It is probably this factor that generates so much disagreement in the literature. Thus, Yamasawa, Cerimele & Serri (1972) in a vast study of fourteen enzymes in sixty-three individuals from 1 month to 90 years of age could only show a scatter of values in a 4 : 1 range and no correlation with age. It seems possible that this range and the disagreement with other authors such as Ribuffo (1967) and Salfeld (1966), who do find differences with age, could be resolved by a study of the kinetics of these enzymes as a function of the subjects' age.

Consequently, workers are turning their attention to determinations of the Michaelis–Menton constant (K_m) of enzymes which is independant of enzyme concentration (Cotton & Mier, 1972). Holla *et al.* (1972) have recently demonstrated that the kinetics of the enzyme cyclic- adenosine-monophosphate- phosphodiesterase (cAMPP) shows a regular difference between the limbs and the trunk, and Cotton & van den Hurk (1972) have also shown a site variation in the K_m for the hydrolysis of β-naphthyl- acetate in the skin of guinea-pigs.

ENZYME VARIABILITY

Isoenzymes

The demonstration that many inborn errors of metabolism can be associated with kinetic abnormalities in specific isoenzymes (Harris, 1970) has stimulated workers to study isoenzymes in the skin. Halprin, Ohkawara & Fukui *et al.* (1968) have made an extensive study of isoenzymes in the epidermis. Uitto, Turto & Lindy (1972) carried out an admirable study of lactic dehydrogenase (LDH) in human skin and showed that a marked decrease with age occurs but that the isoenzyme pattern remains constant.

The isoenzyme pattern, however, was characteristic for skin in that two of the five isoenzymes (LDH and LDH_2) were missing from the epidermis, even though they were present in the dermis. Davidson *et al.* (1963) demonstrated that cultures grown from single skin cells, obtained from human females heterozygous for glucose-6-phosphate dehydrogenase (G6PD) variants, differed in electrophoretic patterns; that is to say, morphologically identical skin cells within one individual may have different isoenzyme ratios.

The possible significance of isoenzyme alterations during the development of cells is indicated in an article where the authors studied the isoenzymes of hexokinase as a function of the development of red blood cells (Gerber, Schultze & Rapoport, 1970). These workers found that the immature cells had a high-K_m enzyme that was more suitable for the environment of the immature cells than the low-K_m enzyme by which it was replaced in the adult cells. It would be of the greatest interest to know if such a phenomenon occurs through the horizontal layers of the epidermis.

DISEASE VARIABILITY

Sites and ages of predilection

In the article of Holla *et al.* (1972) *cAMP* phosphodiesterase was shown to have a lower K_m in the areas of predilection for atopic lesions, the limbs, than in the trunk where lesions are less common. Baillie, Calman & Milne (1965) have demonstrated hydroxysteroid dehydrogenases in the skin and have shown that, histochemically, their reactions are largely limited to the areas of acne predilection. Cotton, Van den Hurk & Van den Staak (1972) have recently shown an association of glucose-6-phosphate dehydrogenase kinetics with the sites of predilection of lichen planus. There are, however, no reports connecting disease with site, age, isoenzymes and enzyme kinetics as yet in the dermatological field..

It is well known to embryologists (Balinsky, 1965) that enzymes may appear during a stage of development, act for a few hours or days, and then disappear; an abnormality in such an enzyme could well account for those skin diseases which have a specific age distribution.

The data briefly discussed here underline the advantages that dermatologists possess. The biochemical approach is sound, the diseases can, in most cases, be accurately diagnosed and investigative techniques are established and standardized. The combination of the keratome for horizontal stratification and the Lowry techniques for histological sampling, make it possible to work with relatively pure cell types, and the Lowry micromethods render enzyme kinetic determinations possible on such small samples. Micro-electrophoretic techniques are well established for the demonstration of isoenzymes, and radioactive and fluorescent assays can be used to determine the kinetics of such isoenzymes. The experimental dermatologist works with the most readily available and most delicately graded and differentiated of tissues; the former makes the elucidation of the mechanisms of the latter an exciting possibility.

Department of Dermatology
University of Nijmegen
Nijmegen
The Netherlands

D.W.K. COTTON

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British Association of Dermatology, London 1972

ANNUAL GENERAL MEETING

The 52nd Annual Meeting of the British Association of Dermatology was held at the Royal College of Physicians, London, 6–8 July 1972, under the Presidency of Dr F.Ray Bettley.

At the Annual General Meeting the following officers were elected for 1972–73:

President: Dr G.A.Hodgson

Immediate Past President: Dr F.R.Bettley

Vice-President: Dr H.J.Wallace

Treasurer: Dr O.L.S.Scott

Editor: Dr A.J.Rook

Secretary: Dr J.S.Pegum

Ordinary Members of the Committee: Dr I.W.Caldwell, Dr Etain Cronin, Dr K.D.Crow, Dr C.N.D. Cruickshank, Dr M.Garretts, Dr R.R.M.Harman, Dr A.Lyell, Dr J.B.Lyon, Dr R.S.Wells.

Willan Librarian: Dr P.W.M.Copeman.

Honorary Foreign Member: Professor W.Schneider of Tübingen.

Ordinary Members: Dr Grace Allen, Dr R.S.Chapman, Dr T.A.J.Dawson, Dr M.English, Dr O.A. Finn, Major Colin Hindson, Dr K. Keczkes, Dr M.I.S.Keir, Dr S.A.Khan, Dr R.Marks, Dr R.D.B. Peachey, Dr C.A.Ramsay, Dr E.H.Wyatt.

Overseas Members: Dr H.W.Buck, Dr Walter Gordon, Dr E.J.W.Mitchell.

It was announced that the next Annual General Meeting would be held in Cardiff on 12–14 July 1973. The next Winter Meeting of the Investigative Group would be held in London on 3 February 1973.

The guests this year included members of the German Dermatological Society.

The following papers were read:

S.Selwyn: Topical Steroids, Antibacterials and Antibiotic Producing Commensals as Factors in the Cross-colonization of Skin Lesions.

Janet M.Higgs and R.S.Wells: Chronic Muco-cutaneous Candidiasis.

John E.White and Susan U.Squier: Folates and Psoriasis.

A.Kobza, C.Ramsay and I.A.Magnus: Sensitivity to the 'Sunburn' Ultraviolet Content of Fluorescent Lighting Tubes.

A.Kobza, C.Ramsay and I.A.Magnus: Failure of Oral Carotene as a Protective Agent in Sensitivity to Long Wave Ultraviolet and Visible Radiation in Solar Urticaria and Actinic Reticuloid.

E.Wilson Jones and Garcia e Silva: Kyrle's and Flegel's Disease.

Mark Hewitt, Ian Barrow, Douglas Miller and Frank Turk: How Important are the Acarine Fauna of the Personal Environment in Skin Disorders?

Rodney P.R.Dawber, Charles D.Calnan and J.Alan Swift: Matting of Hair. Clinical and Scanning Electron Microscopic Findings in Two Cases.

G. Stüttgen: Toxic Epidermal Necrolysis Provoked by Barbiturates.

F.Schellander and R.Marks: The Dermal Influence in Epidermal Hyperplasia.

R.Marks and G.C.Wells: Lichenification: Morphodynamic Correlates.

P.P.Seah, R.M.H.McMinn and Lionel Fry: The Significance of Lymphocytic Infiltration of the Small Intestine in Dermatitis Herpetiformis.

D.J.Riches and Lionel Fry: Ultrastructural Observations on the Dermal–Epidermal Junction in Uninvolved Skin in Dermatitis Herpetiformis.

J.L.Verbov and R.S.Wells: Hypohidrotic Ectodermal Dysplasia.

D.Pongsehirun, R.Marks, E.Wilson Jones and A.Du Vivier: Superficial Fatty Naevus.

Margaret F.Spittle and John E.White: Basal Cell Carcinoma after X-ray Epilation of the Scalp.

R.M.MacKie, A.J.Cochran, W.G.S.Spilg and C.E.Thomas: Studies of Cell Mediated Immunity to Tumour Associated Antigens in Patients with Malignant Melanoma.

E.Abell and Martin M.Black: Comparative Light Microscope and Direct Immunofluorescent Findings in the Differential Diagnosis of Inflammatory Facial Dermatoses.

T.Nishikawa, R.Marks and B.Bhogal: Epidermal Cell Migration. The Effect of Cytochalasin, Tri-iodothyronin and Corticosteroids *in Vitro.*

O.Braun-Falco: Benign Giant Cell Synovialoma.

M.W.Greaves and I.B.Sneddon: Local Heat Urticaria—a Clinical and Pharmacological Study.

E.Abell and P.D.Samman: Intradermal Triamcinolone Treatment of Nail Dystrophy.

E.Wilson Jones and G.C.Wells: The Histogenesis of Syringocystadenoma Papilliferum.

The Clinical Meeting took place at the Middlesex Hospital on 8 July.

The President's Reception was held in the United Service Club, Pall Mall, and the Annual Dinner at the Royal College of Physicians.

The Dowling–McCaw Golf Cup, played for at the Royal Mid-Surrey Golf Club, Richmond, was won by Eric Waddington.

The competition for the Bowers-Sneddon Sailing Trophy, on the Welsh Harp in Northwest London, was won by Roger Harman and John White.

The meeting was outstandingly successful. The President conducted the proceedings with wit, tact and vigour and the arrangements by the Local Secretary, Dr R.S.Wells, were faultless.

A.J.R.

Correspondence

HYPOHIDROTIC ECTODERMAL DYSPLASIA: UNUSUAL PALMPRINT IN A HETEROZYGOTE

SIR, I enclose an enlarged photograph (Fig. 1) of a plastic imprint of a portion of the left hypothenar area (just proximal to the distal transverse flexion crease) of an infant female aged 2½ months. The interesting finding from an anatomical viewpoint is the presence of pores not only along the apices of ridges but also in reasonable numbers both between (i.e. in the furrows or sulci) and on the margins of the ridges. The child concerned is the daughter of a male with hypohidrotic ectodermal dysplasia and is thus an obligatory heterozygote in view of the X-linked inheritance of that condition. However, I have seen pores in reasonable numbers between ridges on one other occasion, in a normal female aged 4 years, and the finding, although very uncommon is unlikely to be related to hypohidrotic ectodermal dysplasia.

Both Harold Cummins and Sarah Holt, world authorities on epidermal ridges, have not seen anything like this imprint before and it is clearly not an artefact although the imprint is not too clear in parts. A skin biopsy would be interesting of course, to see the anatomical position of the pores, but is not ethical in an infant of this age.

Professor Cummins (1972) commented that embryology clarifies the unexpected location of sweat pores. Embryologically the sweat glands arise from elevations (glandular folds) of the deep surface of the epidermis, and the duct of a sweat gland passes upwards through a glandular fold to appear as a pore on the surface at the apex of a ridge. Another generation of such elevations is produced by epidermal proliferation from the sides of the initial ones and from the intervals between them (interstitial ridges). The interstitial ridges do not show sweat gland anlagen (Hale, 1952). In Fig. 1, pores situated in

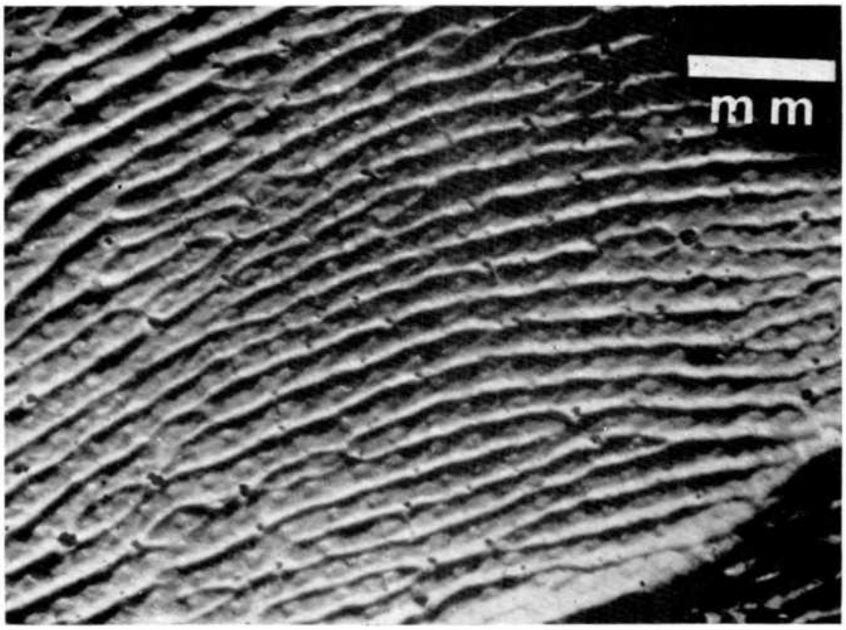

FIGURE 1. Female aged 2½ months. Palmar imprint showing sweat pores along, between, and at the margins of ridges. (× 20)

furrows (sulci) are related to the sites of interstitial ridges whilst the pores on the sides of ridges are a retention of embryonic relations. It is possible that differential growth might later bring some pores at the ridge margins to the ridge summits in the child described but if this is so I would have expected marginal pores to be a common finding in imprints in children, which they are not.

JULIAN VERBOV

Department of Dermatology
Liverpool Royal Infirmary
Liverpool L3 5PU

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GLUCOSE METABOLISM IN PSORIATIC EPIDERMIS—LOCALIZATION OF ALTERED ENZYME ACTIVITIES

SIR, Jarrett (1971) reported the histochemical detection of glucose-1-phosphate dehydrogenase in epidermis, as reflected in the reduction of nitro-blue tetrazolium (NBT) in the presence of G1P. This enzyme has not to our knowledge been unequivocally demonstrated in any tissue, and it is therefore appropriate to examine other mechanisms for reduction of NBT in the presence of G1P. One such pathway might be conversion of the substrate to uridine diphosphate glucose (UDPG) which is then oxidized by the appropriate dehydrogenase to yield UDP-glucuronate and NADH, and subsequent transfer of hydrogen from the reduced pyridine nucleotide to NBT. However, the activity of UDPG-DH in epidermis is low (Fukui & Halprin, 1970), and one would expect it to be localized in the dermis where it provides precursors for mucopolysaccharide synthesis. The major epidermal fate of the glucose moiety of UDPG appears to be incorporation into glycogen.

A highly probable fate of exogenous G1P is conversion to G6P by the ubiquitous enzyme, phosphoglucomutase (PGM). The G6P thus formed can undergo several transformations, including that catalyzed by glucose-6-phosphate dehydrogenase (G6P-DH). Thus, the reduction of NBT in the presence of G1P probably represents the combined actions of PGM and G6P-DH. The former enzyme (PGM) is usually not rate-limiting in systems utilizing glucose phosphates, as illustrated by Jarrett's observations with mouse tail epidermis: reduction of NBT in different areas was essentially the same with G6P or G1P as substrate. However, a significant difference was noted in human psoriatic skin. The data in Table V of the report cited reveal that no NBT reduction occurred with either substrate in the lower zone of normal or psoriatic epidermis or in the transitional zone of normal epidermis, after 24 min incubation. On the other hand, the transitional zone of psoriatic tissue exhibited G6P-DH activity after 24 min, whereas the demonstration of activity with G1P required 2 h incubation. These results imply a relative lack of PGM in the transitional zone, which if true, represents a unique observation that may help elucidate the biochemical aspects of psoriasis. For example, the absence of glycogen in the epidermal transitional zone may be due to decreased G1P concentration and consequent reduction in UDPG synthesis.

The shift toward G6P oxidation *via* the hexosemonophosphate pathway (HMP) in psoriatic epidermis is understandable on the basis of the relative increases in activities of G6P-DH and 6-phosphogluconate dehydrogenase (6PG-DH) that have been observed by several investigators. Jarrett's detection of elevated G6P-DH in the transitional zone provides a valuable clue to the anatomic locations of the shift in glucose metabolism, and it would be of considerable interest to learn whether the second enzyme (6PG-DH) also exhibits elevated activity in this region. This could be ascertained by use of the appropriate substrate, 6-phosphogluconate (Sigma). Another study is suggested in a purely speculative vein, with the rationale that it would be easy to perform and might provide information concerning a third transformation of G6P (in addition to HMP oxidation and conversion to G1P). This would involve localization of NBT reduction in psoriatic epidermis in the presence of fructose-6-phosphate, as a measure of phosphohexose isomerase activity.

In conclusion, the technique developed by Jarrett for quantitating and localizing the activity of dehydrogenases in skin sections offers considerable potential for further study of carbohydrate metabolism in psoriatic tissue. A word of caution is injected in that unless specific inhibitors are employed to verify that the histochemically-observed effect is indeed a result of the assumed dehydrogenase reaction, the experimental data must be regarded as tentative. It is to be hoped that results obtained with Jarrett's technique may be further validated with biochemical assay of enzyme activities in microdissected areas of skin sections. Tentative validation of his procedure may be inferred from the observation of Hammar (1971) who reported that the assay value of G6P-DH activity in subcorneal psoriatic tissue was about 30% higher than that in the basal epidermis.

JOHN A. JOHNSON
RAMON M. FUSARO

Departments of Dermatology and Biochemistry
University of Nebraska Medical Center
Omaha
Nebraska 68105
U.S.A.

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GLUCON METABOLISM IN PSORIATIC EPIDERMIS

SIR, The observations of Dr Johnson and Dr Fusaro concerning the specificity of the glucose-1-phosphate dehydrogenase reported in the hair cortex and tail scale epidermis of the mouse are of interest. They suggest that the substrate may be altered by endogenous phosphoglutomutase into glucose-6-phosphate which is then acted upon by glucose-6-phosphate dehydrogenase. Since writing this paper (Jarrett, 1971), further work on dehydrogenase systems in the epidermis has indicated that the reaction for glucose-1-phosphate dehydrogenase in the hair cortex and the tail scale epidermis of the mouse is indeed non-specific. However, the reason for this appears to be that tetrazolium salts are reduced non-specificially at these sites in the mouse. A reaction can be obtained with buffered nitro-blue tetrazolium alone at pH 7·4, without either the substrate or NADP. This non-specific reaction, which is usually obtained at a more alkaline pH with tetrazolium salts plus NAD (or NADP), has been termed by Zimmerman & Pearse (1959) as 'nothing dehydrogenase'. In this context it is of interest to note that in the follicular regions of the mouse tail, in distinction to the tail scale regions, an oxidase system is present which darkens 5-hydroxy-tryptophane. These non-specific reactions clearly distinguish these two sites of the mouse tail epidermis (Jarrett, 1972)

In view of these findings one must agree that the reaction reported previously does not establish the presence of glucose-1-phosphate dehydrogenase in mouse skin. However, the reaction for glucose-1-phosphate dehydrogenase was negative in human skin both from normal idividuals and from psoriatic plaques. In normal human skin the reaction for glucose-6-phosphate dehydrogenase was also negative, but a positive reaction was obtained in the transitional zone of psoriatic epidermis. The latter cannot be due to 'nothing dehydrogenase' because of the negative results with glucose-1-phosphate. The increased activity of glucose-6-phosphate dehydrogenase in psoriatic skin has already been reported biochemically (Rassner, 1965, 1966).

Their further comments, together with the suggestion that specific inhibitors should be used, are valid, especially in view of the finding of 'nothing dehvdrogenase' in certain skin sites. Further work is proceeding and the results will be reported later.

A. JARRETT

University College Hospital Medical School
London, W.C. 1

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TOPICAL AMINOGLUTETHIMIDE IN HIRSUTISM

SIR, Idiopathic hirsutism is one of the more difficult problems for dermatologists to treat. Since the mechanism of this condition is thought to be an increased end organ sensitivity to androgen we wondered if aminoglutethimide (Elipten), a potent inhibitor of steroid biosynthesis interfering with the conversion of cholesterol to pregnenolone (Dexter *et al.*, 1967), could influence the clinical state. During the past

TABLE I

	Weight of hair (mg/unit area) on side treated with aminoglutethimide	Weight of hair (mg/unit area) on side treated with base alone
Month 1	88·8 ± 2·2	89·5 ± 3·6
Month 2	60·7 ± 1·1	60·9 ± 2·2

18 months we have investigated the effect of topical aminoglutethimide on eleven patients with idiopathic hirsutism.

On the first and subsequent monthly visits the hairiness was assessed clinically and a constant area of the anterior tibial surface of both legs was shaved and the shavings weighed; blood was also taken for plasma cortisol estimation. The patients were supplied with two tubes of ointments, one to be applied to each leg twice daily for 2 months. The active ointment contained aminoglutethimide in fatty alcohols and propylene glycol (w/v 5%) and the other contained fatty alcohols and propylene glycol.

There was no visible difference in the hair growth on either leg and Table 1 shows that there was no significant difference between the weighings. Our findings of a lack of effect and our conclusions are similar to those of Burton, Cartlidge & Schuster (1972) who found that aminoglutethimide did not influence sebum excretion rate in patients with acne. We do not know whether the lack of effect was due to the inadequate percutaneous absorption of the drug or whether aminoglutethimide acts too early in steroid biosynthesis to have an effect.

General Infirmary
Leeds

W.J.Cunliffe
M.Williams

ACKNOWLEDGMENTS

We wish to thank the Board of Governors, Leeds General Infirmary, and the Wellcome Trust for financial help, and to Dr D.M.Burley of Ciba Laboratories for the supply of pure aminoglutethimide powder.

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Book Reviews

Systematische Dermatologie und Grenzgebiete. Vol. 2. W.Gertler (1972) Leipzig: Thieme. Pp. 643–1377, 181 figures. Price £14.20.

The first of the three volumes of this encyclopaedic dictionary of dermatology was reviewed in this Journal in April 1971. The second volume covers eczema, including occupational dermatoses, seborrhoea, acne and rosacea, psoriasis, lichen planus, and parasitic, bacterial and viral infections. The tightly concise style and the card-index presentation enable the author to compress a vast amount of information into 734 pages. The long and useful lists of references which end each section of the book are understandably predominantly to German language journals, but they include many recent references to other journals including British and American. However, the coverage of the literature is often curiously patchy and in some chapters as in that on acne, even when some of the important recent foreign papers are listed they appear not to have influenced the author's views on pathogenesis, which many would consider outmoded. The account of scabies is adequate but the references omit the three standard monographs on this disease, one Argentinian, one British and one Danish.

Many dermatologists will be surprised and dismayed to see inorganic arsenic and mercury ointment recommended for the treatment of psoriasis. Elsewhere in the book treatment procedures advocated differ considerably from those in common use in Britian and the United States.

Because this book is both comprehensive and compact it deserves a place in the library of the larger departments of dermatology. The fact that the references listed sometimes cover a somewhat different range from most textbooks in use in English speaking countries is perhaps an asset.

ARTHUR ROOK

Ovulationschemmer in der Dermatologie. H.ZAUN (Ed.) (1972) Stuttgart: Thieme. Pp. 67+vii. Price £3.15.

Professor Zaun of Homburg/Saar has edited the proceedings of a symposium, to which he and ten colleagues, mainly from South West Germay, contributed papers on the dermatological applications and implications of the contraceptive pill. Most of the eleven papers combine reports of their authors' own investigations and observations with a comprehensive review of the international literature. Their publication is warmly to be welcomed for they present clearly and concisely much important information gathered from widely scattered sources.

Tronnier discusses his experimental studies on the effects of the 'pill' on melanogenesis, light sensitivity, sebaceous and sweat glands. Zaun writes on the influence of the 'pill' on hair growth and its use in the treatment of some forms of alopecia. Husmann and Ludwig consider the therapeutic effects of the 'pill' in hirsutism, and in seborrhoea and acne, respectively. Zaun reviews the rarer indications for the 'pill', such as Fox–Fordyce disease. Klostermann summarizes the cutaneous side-effects of the 'pill', other than porphyria cutanea tarda, the subject of a separate paper by Albers. Candidiasis is covered by Meinhof, and vaginal discharges by Nussbaum. Schneider writes of the pathophysiological and Jacobs and Heinen of the statistical and clinical aspects of thromboembolic complications.

This is an extremely useful book but serious criticisms must be made of some chapters. Most authors emphasize the obvious differences in effects, desired or undesired, according to the composition of the pill prescribed; for example Ludwig found that in the control of seborrhoea, chlormadinone/mestranol was about twice as effective as lynestrinol/mestranol. Other authors discuss the quantitatively and qualitatively diverse formulations available as if they were a single consistent pharmacological agent. A second criticism is that some therapeutic trials appear not to have been matched with untreated controls. A third criticism applies to Husmann's paper on the treatment of hirsutism. He treated with chlormadinone acetate 3·0 mg and mestranol 0·1 mg thirty-nine women with various forms of hirsutism. Hair growth was 'normalized' in twenty-seven of the patients and significantly improved in a further three. Such exceptionally good results call for a very much more detailed account of the chemical and biochemical findings, but no doubt Dr Husmann plans to publish this elsewhere.

ARTHUR ROOK

An Introduction to the Diagnostic Histopathology of the Skin. J.A.MILNE (1972) London: Arnold. Pp. 363, illustrated. Price £9.00.

To those dermatologists long familiar with the several well established American textbooks on histopathology of the skin, the appearance of a British counterpart must arouse no small measure of excitement and interest.

Professor Milne's new book takes its origin in an annual course in skin histopathology which he has conducted in Glasgow, catering for the needs of trainee dermatologists and general pathologists. The resulting account should prove equally satisfying to both groups, and in many places clarifies various points which the general pathologist finds confusing, and which the dermatologist in his occasional isolation, often takes for granted.

After a short introductory chapter on biopsy technique, and a detailed review of the microstructure of normal skin, the author gives a well illustrated account of the various descriptive terms peculiar to cutaneous pathology.

Later chapters deal systematically with the many skin disorders normally subjected to biopsy, and an appendix gives a useful outline of laboratory methods and staining techniques which can be used to demonstrate specific histological features.

Space does not permit a detailed analysis of the various sections, but the reader cannot but be impressed by the excellent balance achieved throughout the book, and the immense care which has been taken in dealing both with the common disorders such as eczema and psoriasis, and with the more exotic dermatological curiosities. Each condition is illustrated by well chosen photomicrographs of outstanding quality, accompanied by legends which are unusually detailed and helpful.

By omitting the usual voluminous lists of references, the author has been able to produce a very personal and lucid account of the subject. For the student beginning his study, and for the general pathologist coping with a wide variety of pathological conditions, a detailed bibliography can be superfluous. The space thus saved can be put to much better use, as in this book, by providing a more detailed pictorial cover, and a more practical analysis of the histological features. For the advanced student these references can be readily obtained from one of the larger text books.

It is difficult in a book of such overall excellence to single out any part for particular commendation, but the sections on granulomatous inflammation of the skin and bullous disease are well covered, and that on vasculitis is a refreshing simplification of previous confusing accounts. Attention is drawn to the value of some of the newer techniques of immunoflorescence and electron microscopy in improving the accuracy of diagnosis in certain conditions, although it is recognized that such facilities are not always readily available.

A short chapter on the pathological features of the various forms of alopecia might be included in a later edition, and the excellent series of photomicrographs of actinic keratoses could be rearranged to keep in better step with the accompanying text. The illustration of an early histiocytoma (Fig. 15.6) is the only photomicrograph in the book which might be considered at first to be misleading, as the majority of such lesions encountered are of a pattern better illustrated by the later sections.

Whilst admirably suiting the needs of general pathologists faced with the reporting of skin sections, this book should quickly establish itself as the best currently available introduction to skin histopathology for the trainee dermatologist, and for those of more mature years with an interest in this absorbing subject.

RONALD W. EMMERSON

Feinstrukturelle Morphologie und Histopathologie der verhornenden Epidermis.

CONSTANTIN E.ORFANOS (1972) Stuttgart: Georg Thieme Verlag. Pp. 204, 82 illustrations. Price £9.75.

The study of the ultrastructure of skin is becoming more and more specialized. This book deals mainly with the fine morphology of the normal and abnormal keratinocyte and mentions only briefly other epidermal components, the basal lamina and the dermis.

C.E.Orfanos summarizes much of his own work over the last 10 years and describes some additional unpublished material. His considerable personal experience is often related to those of others and over 700 references (refreshingly international) are quoted.

Eighty pages are spent on the normal epidermis and the rest on pathological changes which are dealt with under general (spongiosis, parakeratosis and acantholysis, etc.) and specific disease headings. There are sections on dermatitis, lichen planus, ichthyosis, psoriasis, bullous disease and some epidermal tumours (excluding squamous cell carcinoma and pigmented tumours).

The text is illustrated with over eighty plates, all of which are clearly labelled. Most are standard transmission electron micrographs but various cytochemical procedures including proteolytic digestion of epidermal cells, sodium thioglycolate treatment of hard keratin, acid phosphatase and cholinesterase preparations are also demonstrated. There are a few excellent scanning electron micrographs. Frequent tables and simple line diagrams summarize the author's views and the latter are presented in a concise enough fashion for further workers to prove right or wrong. It is of interest that the author adheres to the view that damage to the tonofilament–desmosomal complex is the prime event in the true acantholytic process.

The micrographs, which with a very few exceptions are the personal work of the author, are clearly reproduced. Although the cellular detail in some of the low power pictures is a little hazy the high magnification plates are most commendable. The acid phosphatase micrographs, however, are far from convincing and the nuclear and cytoplasmic reaction product looks artifactual and non-specific even though in the text the author accepts the appearance as significant.

This book deserves to sit on the same library shelf as the Zelickson, Hashimoto and Lever and Breathnach tomes but the number of English speaking dermatological electron microscopists who understand German must be very small (hence the tandem review here). It seems a great pity that there is not even a summary in English at the end of each section.

P.J.HARE
J.A.A.HUNTER

Chemistry of Fungicidal Action. No. 10 in 'Series' Molecular Biology, Biochemistry and Biophysics. R.J.LUKENS (1972) London: Chapman and Hall. Pp. 136, 8 figures. Price £5.00.

This book examines the chemical and physical properties of fungistatic and fungicidal agents and their reactions with fungi.

Fungitoxicity is defined as the ability of a chemical to interfere in an adverse way with the vital functions of a fungus by physiochemical means. Responses of fungi to toxic agents as assessed by inhibition of growth, spore germination, respiration and sporulation are enumerated in a practical chapter on measurement of fungicidal action, which also includes a description of dosage-response phenomena suitable for obtaining linear curves for computing indices of fungitoxicity.

Methods of application of fungicides, factors concerning the dispersal, deposition, retention, persistence and formulation of antifungal agents are fully described. The paths of migration of a fungicide to sites of action where reactions with cellular constituents inhibit further growth of the fungus are discussed. The latter part of the book deals with the various component structures of the fungus and how fungicides can affect these constituents. Thus, the structure of the cell wall, the cell membrane, mitochondria, ribosomes and nucleus and the ways in which toxicants can affect them are detailed separately. In addition, the effect of fungicides on cellular constituents and on enzyme activity is very clearly analysed.

The chemical structure of antifungal compounds and their inhibitory action is discussed and a table is included of some of the more common fungitoxic compounds. Finally, the possible action of the fungus on the fungicide is assessed.

This book is concise, very well set out and extremely readable. A vast amount of valuable data is collated in this small volume and a very useful bibliography included. Although the majority of the specialized information relates primarily to the field of plant pathology, there is a great deal to interest all those concerned with problems of treatment of fungal conditions of man and animals. Griseofulvin and the polyene antibiotics are discussed in the sections dealing with cell wall and cell membrane toxicants respectively. As the author himself states, the principles developed in the book may be applicable to an understanding of the actions of fungicides in the control of disease of plants, animals and of man as well as the action of fungicides in preventing deterioration of material.

Y.M.CLAYTON

Andrews' Diseases of the Skin. ANTHONY N.DOMONKOS (1971). Philadelphia and London: Saunders. Pp. 1027. 1178 illustrations. Price £12.75.

A new 'Andrews' is an octennial event in the world of dermatology since George Clinton Andrews' first *Diseases of the Skin* appeared in 1930 to the great benefit of successive generations of students, doctors of every kind, embryo dermatologists and those of far greater maturity as well. It has always been a very good textbook in the light of the knowledge and philosophy of its successive times and comparisons of edition with subsequent edition thus provide a fascinating march of modern dermatological history. It has never attempted the role of a major work of reference. Anthony N. Domonkos played a large part in producing the fifth edition and has sole responsibility for this new and very much larger and certainly much heavier book. This growth may well in later years be seen as typical of this particular era and I am very much afraid that future historians may also detect that loss of a sense of purpose alleged to be prevalent to-day.

This book is not a major work of reference, though it tells something of many more skin conditions than its predecessors and contains numerous and mostly fairly recent references at the end of each chapter. (It is perhaps small minded to remark that so many of these references are North American but some guidance as to which to look up first would be helpful.) The trouble is that this book is no longer a good text book either. After a shaky anatomical start with a composite description and illustration of glabrous and hairy skin and practically no physiology it becomes an encyclopaedic list of things which go wrong with much too little about how and why and how often. The section on herpes simplex, for instance, describes the localized 'cold sore', covers primary infections under three separate headings, goes back to genital lesions, then on to eczema herpeticum, where it incidentally mentions the immunological status of these various patients, and finally comes back to recurrent localized lesions. By the time we get to treatment the serious infections are forgotten again.

If no logical sense is made of the inter-relationships of mankind and the herpes simplex virus what chance is there of painting an intellectually comprehensible picture of the 'eczemas'? With no preliminary definitions a start is made with 'contact dermatitis' which is divided into primary irritant and allergic. The first example of the former is housewife's eczema and the second diaper dermatitis also, I quote 'known as *napkin psoriasis*' (sic), the third acid burns and the fourth chloraene. Under allergic contact dermatitis we learn about the poison ivy, the primrose (sic) and phytophoto-dermatitis and most of the usual things. We then have 14 pages on drug eruptions of every conceivable type, 6 pages on atopic dermatitis and the last 4½ pages of this exhausting chapter on eczema, nummular eczema bringing up the rear. Seborrhoeic dermatitis comes four chapters later. Histology, here and throughout the book, seems just something else to learn rather than an illuminating part of what is going on.

If one can accept this inconsequential muddle and learn from it, the next objective must be to treat the cases by now diagnosed. Here too I am disappointed more often than not. Exact instructions are too seldom given to make the book a useful therapeutic standby and too often the flat botanical approach which makes so many descriptions rather dead fails to point the way to a more imaginative management of the living patient. Even with more specific remedies however, how many of us would rely on two applications of Eurax to knock out scabies?

There is an immense amount of very good material which must not be ignored, especially in the descriptions of the specific diseases and the new chapters on immunology and tropical dermatoses deserve special mention. Hanseniasis seems a horrible word to European ears and eyes especially when leprosy is almost exclusively used in the text. In the same vein, why mycobactericosis cutis when infections other than by the tubercle bacillus are called 'atypical mycobacteriosis'?

I fear that this great book has, like the dinosaur, evolved too far beyond the point of no return. It is desperately difficult to add without pruning too little, to remain a fundamental teacher while tied to an old and too rigid form and to retain the common touch and the liveliness of true natural history under a mountain of accumulated facts and hypotheses. I would hope that Dr Domonkos, after a well earned rest, would give us from his tremendous energy and ability a brand new text book which will be all his own.

R.D.SWEET

British Journal of Dermatology (1973) 88, 103.

Quarterly Review

CONTACT DERMATITIS XX

Type I and Type IV Allergy in Contact Dermatitis

Turk (1971) has summarized the mechanisms involved in the immune responses concerned with these types of allergy. All the cells involved originate from the bone-marrow. Some pass to the thymus and some to the intestinal lymphoid tissue. Eventually both types are found in the lymph nodes, though in distinctly different areas. Humoral antibodies are produced by plasma cells in the medulla and in germinal centres while those cells involved in cell-mediated immunity are concentrated in the so-called paracortical areas. On the second to fourth day after the epicutaneous application of a strong sensitizer small lymphocytes change into large metachromatically staining lymphocytes in these paracortical areas. On the fifth day these large lymphocytes disappear again leaving only the small lymphocytes.

The relationship of this cell-mediated Type IV allergy to Type I allergy—as exemplified by atopic dermatitis—has been the subject of many experimental and clinical enquiries. For instance, is contact-type sensitivity rare or is it common in patients with the atopic diathesis; or is there a more direct relationship with the broken and inflamed skin of the patient with atopic dermatitis alone? If so, is this related only to medicaments used in treatment? Conversely, is atopic sensitivity more common in patients with contact dermatitis than in the general population? What, indeed, is the role of allergy (if any) in atopic dermatitis? Although the atopic state is characterized by the presence of a high concentration of IgE antibodies, which are probably important in causing urticarial lesions, these do not seem to be directly responsible for the production of eczema. It might be assumed that some unknown cell-bound antibodies might be involved in the eczematous state but an atopic type of eczema occurs in the Wiskott–Aldrich syndrome, where the ability to manifest delayed type is absent.

Recently, Lobitz, Honeyman & Winkler (1972) studied two patients with a severe chronic atopic dermatitis. They found, in both patients, unresponsiveness to delayed-type skin tests such as tuberculin, histoplasmin and trichophytin. Similar findings have been published by Rajka (1967). Lobitz *et al.*, also failed to sensitize their subjects to DNCB and there was a diminished responsiveness of their cultured lymphocytes to phytohaemagglutinin, especially at times when the eczema became very severe. They also found by direct immunofluorescent tests that the medium and small lymphocytes in the peripheral blood were coated with IgE. It is possible that this fixation of IgE to lymphocytes might release an eczema-producing factor, creating spongiosis. Lobitz' study may have a particular bearing on the problem of patients with longstanding and severe atopic dermatitis, the chronicity itself being perhaps due to the phenomen described.

Atopic dermatitis as seen in infants and young adults differs from Lobitz' cases when it presents as a universal or extensive enzema. Certainly atopic and contact dermatitis are not mutually exclusive. In a study carried out by the International Contact Dermatitis Research Group (Cronin *et al.*, 1970), 233 of 4000 patients patch tested had atopic dermatitis. The incidence of positive reactions for those with atopic dermatitis, seborrhoeic dermtitis and nummular eczema was similar. The low incidence of the positive reactions is surprising in view of the long duration of contact with topically applied medicaments. For these are very common sensitizers in patients with stasis dermatitis or ulceration.

It is known that allergic contact dermatitis depends to some extent on genetic predisposition and that this is very much the case in atopic dermatitis. Forsbeck, Skog & Ytterborn, (1968) patch tested 101 pairs of twins and found no correlation between atopy and contact sensitivity but in their later studies performed in Stockholm some results appeared to be in conflict with these findings (Forsbeck Skog & Ytterborn, 1971). Here the material subjected to study comprised patients with positive patch tests. Atopy was no more common among these than in the normal population. On the other hand, the relatives of patients with contact dermatitis had a higher frequency of atopic reactions than expected. This is the most decisive clinical evidence we have of a relationship between atopy and contact sensitivity.

In some clinical material contact with proteins appears to be a causative or provoking factor of dermatitis in atopic subjects. Nexmand (1948) noted that some patients sensitive to horse dander had a flare of their eczema on contact with horses and some patients with atopic eczema who are sensitive to the house-dust mite improve dramatically after treatment of the home with insecticides. In 1968 Malten reported a group of 'eczema hybrids,' i.e. patients with concomitant atopic and contact sensitivities. These form an interesting group but appear to be quite uncommon except in certain occupations. During a review of veterinary surgeons with occupational dermatitis we became aware that they, as well as milkers, may be sensitive to cow's hair (Hjorth, 1967). Irritant dermatitis is common in both these occupations and may be a prerequisite. Similarly, dermatitis in bakers may occur in atopic individuals and here contact with flour seems to be a more decisive eliciting factor than inhalation. Atopic housewives may also develop a flare of eczema after contact with fish or eggs.

Most veterinary surgeons seen by dermatologists have allergic contact dermatitis caused by contact allergens alone or contact and protein allergens; among the former antibiotics are important, among the latter, cow's hair. But it is of interest to note that, despite heavy exposure to pollen, no cases of hayfever were seen in this study, though some of them develop dermatitis if they touch a cow.

A further specialized groups of patients—chefs and sandwich-makers—have recently been studied (Hjorth, 1972). Occupational dermatitis was, rather surprisingly, found to be very common indeed. Patch tests were commonly negative and it was apparent that they suffered, not from an irritant dermatitis but from a contact dermatitis from handling particular foods such as fish and prawns.

It is clear that further studies are required before we can form a clear picture of the relationship between these two types of allergic response.

N. Hjorth

Department of Dermatology
Amtssygehuset, DK. 2900, Hellerup, Denmark

D. S. Wilkinson

Wycombe General Hospital
High Wycombe
Bucks.

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British Journal of Dermatology (1973) 88, 106.

News and Notices

Advanced Seminar in Dermatology

This Annual Seminar is open to consultants and senior registrars in dermatology. It will be held from 16 to 19 April 1973 inclusive at The Institute of Dermatology and St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London, WC2H 7BJ. The course will include histopathology, immunology and photobiology.

Applications should be sent to the Dean before 1 February 1973.

APPOINTMENTS

Dartford, Cray Valley and Sevenoaks Group

Dr Angus McDonald has been appointed Consultant Dermatologist to the Dartford, Cray Valley and Sevenoaks Group of Hospitals. Dr McDonald qualified M.B., Ch.B. Edinburgh in 1959. He became a member of the Royal College of Physicians of Glasgow in 1963 and of London in 1965. His junior hospital appointments were in Carlisle, Edinburgh and at St Leonard's Hospital, London. From 1966 he has been Senior Registrar at St Mary's Hospital, London. He has a large number of publications to his credit, notably investigations into psoriasis and the effects of retinoic acid in treatment of this condition.

Enfield Group of Hospitals

Dr John James Ryan Almeyda has been appointed Consultant Dermatologist to the Enfield Group of Hospitals. Dr Almeyda qualified M.B., B.S., from St Bartholomew's Hospital Medical College in 1960, having obtained the Herbert Patterson Gold Medal in Biochemistry. He became M.R.C.P. in 1966. After doing 2 years in pathology at his own hospital he eventually became Registrar and later Senior Registrar in Dermatology at the London Hospital. Dr Almeyda is especially interested in drug reactions and has written a number of articles on this subject and especially on the toxic effects of methotrexate.

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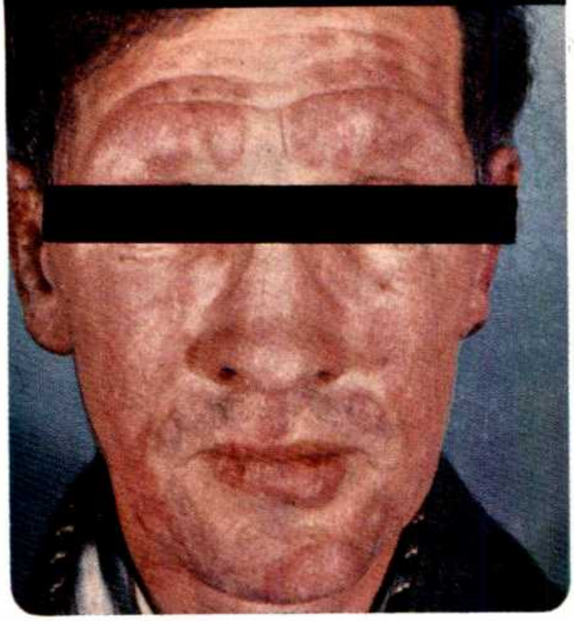

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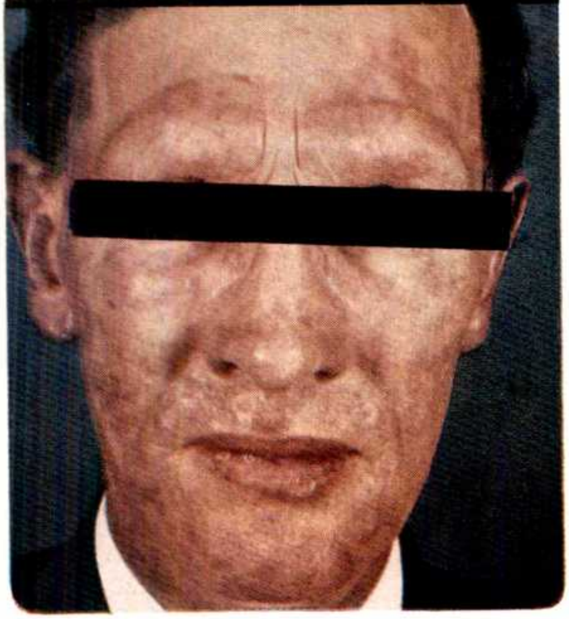

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Journal of the Society of

Cosmetic Chemists

of Great Britain

Edited by J. M. Blakeway

VOL. 24 NO. 1 JANUARY 1973

M. CAMBRAI AND H. DO-LINH. Phoreography, a new method of determining the effect of uv radiation on skin.

C. E. JOHNSON AND S. SHUSTER. The use of partial sweat duct occlusion in the elucidation of sweat duct function in health and disease.

I. M. GIBSON. The evaluation of hand-care preparations.

L. GEOFFREY HOLE. Sweat disposal from footwear and health and hygiene of foot skin.

H. McG. SCOTT. Methods for counting and testing for micro-organisms in raw materials, topical and oral products.

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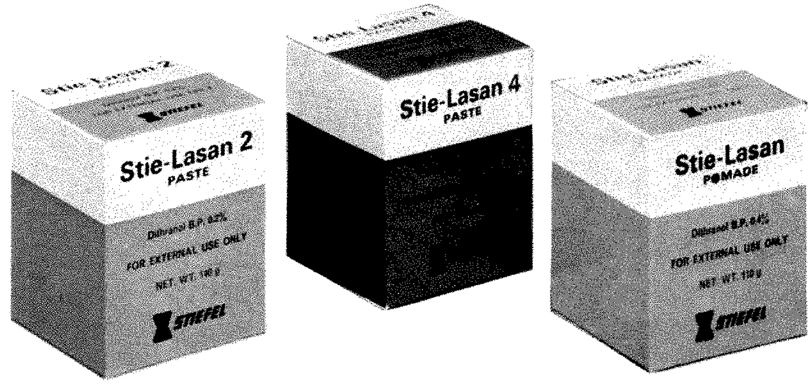

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microgram(s) (10^{-6} g)	µg	cubic millimetre(s)	mm^3	micrometer(s)	µm
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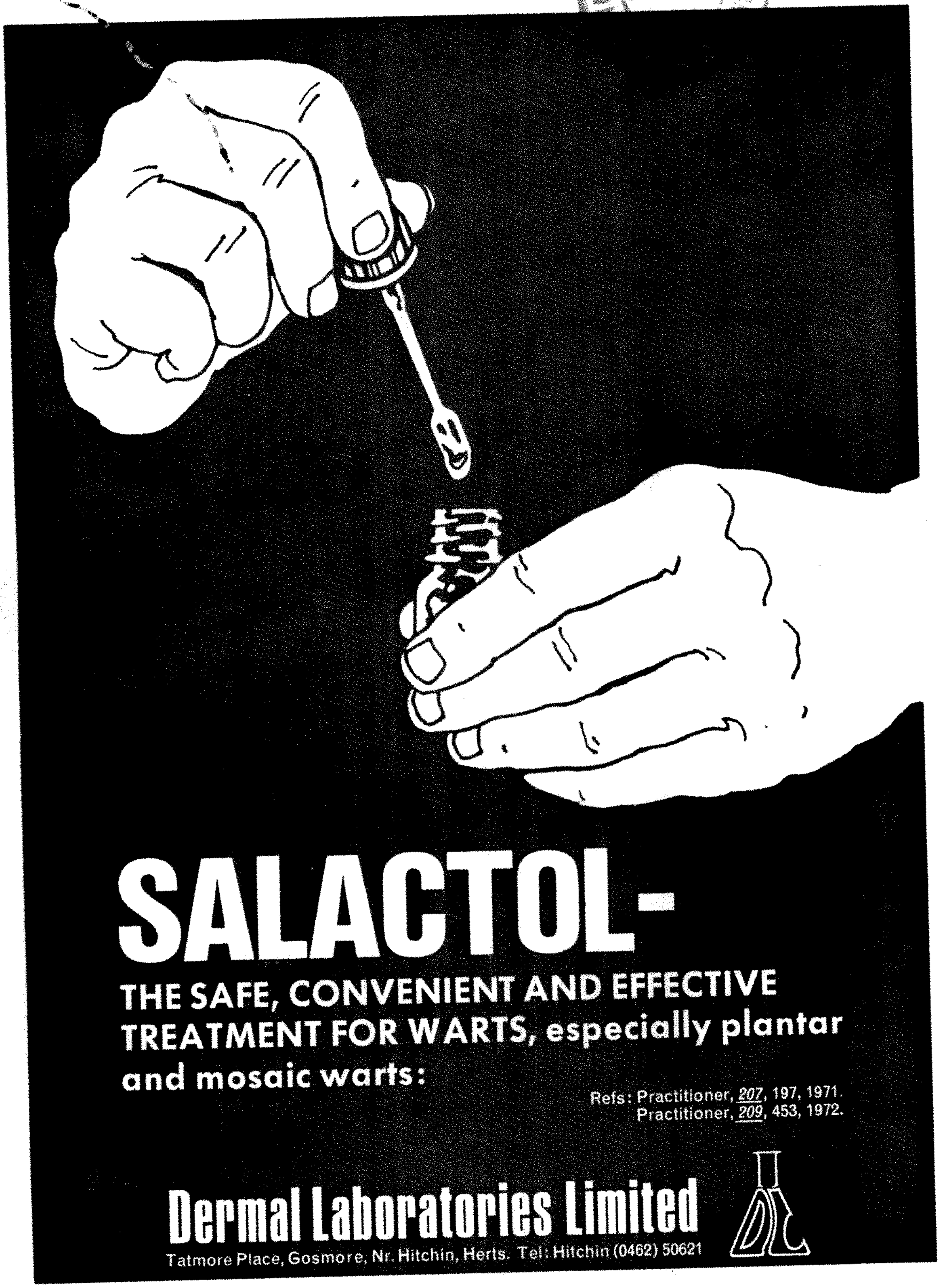

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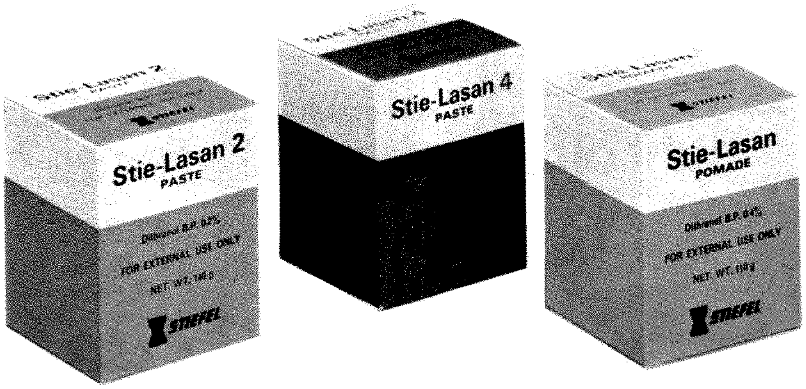

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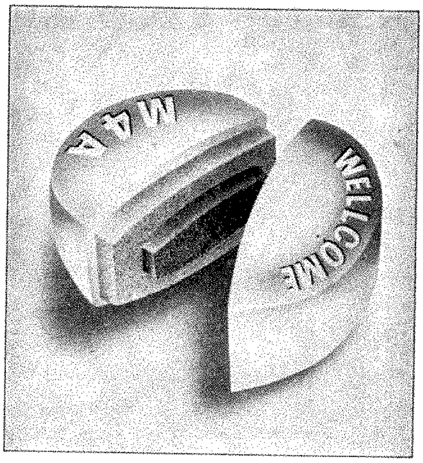

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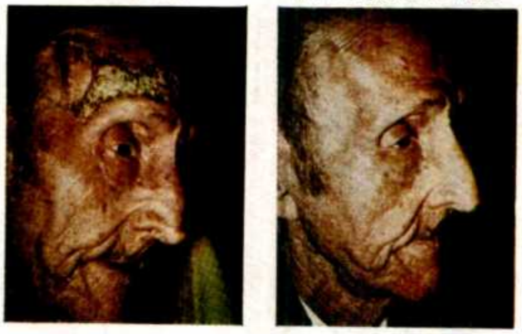

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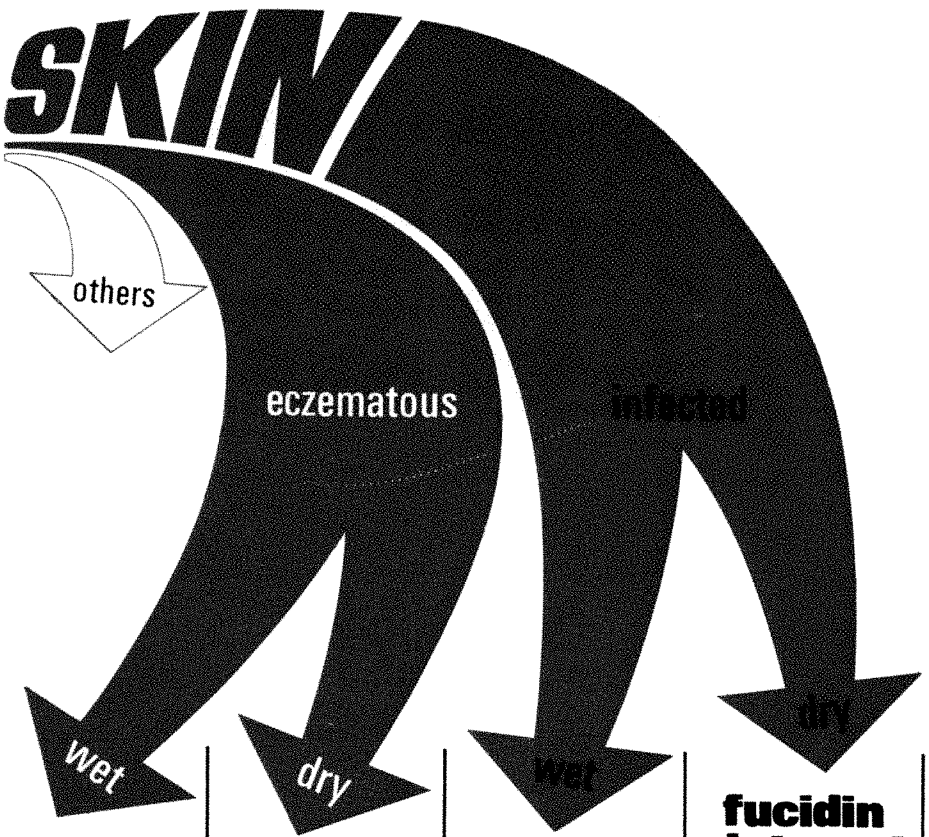

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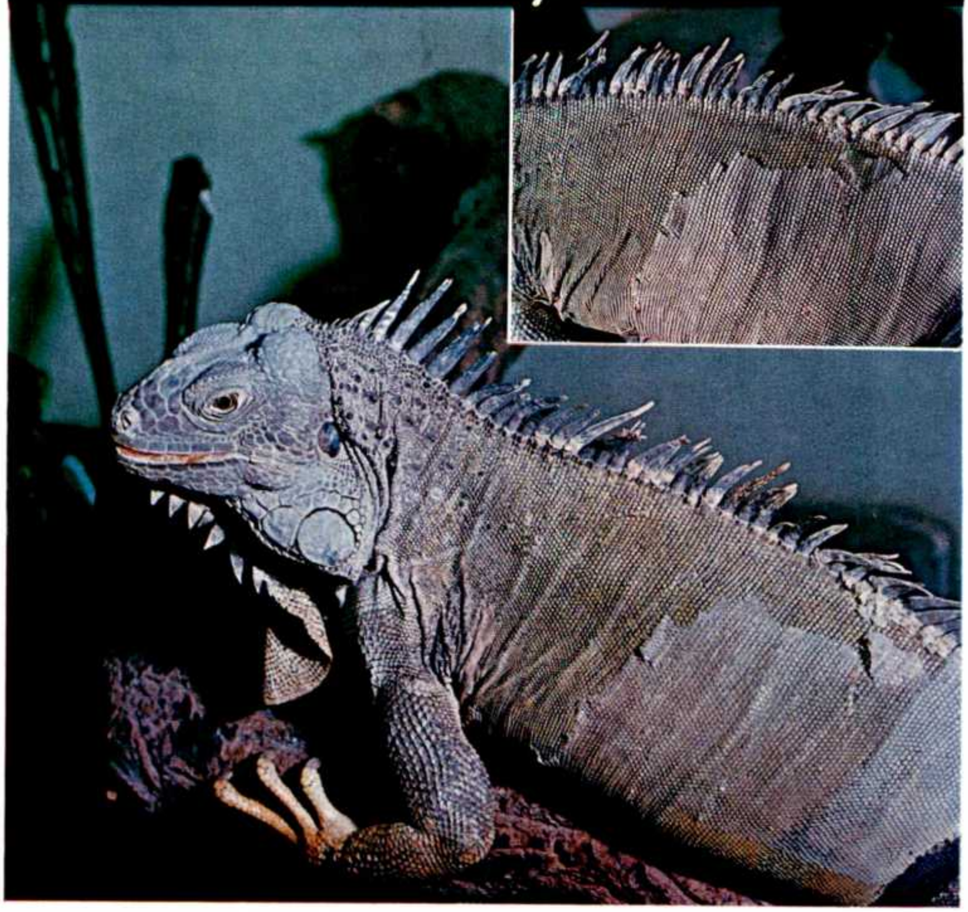

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Clinical and Laboratory Investigations

Scleromyxoedema (lichen myxoedematosus) associated with a paraprotein, IgG_1 of type kappa

R.F.M.LAI A FAT,* D.SUURMOND,* J.RÁDL† AND R. VAN FURTH‡

* Department of Dermatology, University Hospital, Leiden, † Institute for Experimental Gerontology TNO, Rijswijk, The Netherlands, and ‡ Department of Microbial Diseases, University Hospital, Leiden

Accepted for publication 9 August 1972

SUMMARY

A case of scleromyxoedema (lichen myxoedematosus) associated with an IgG_1, type kappa paraproteinaemia is reported.

Culture of bone marrow material demonstrated that this tissue synthesized monoclonal IgG (subclass $\gamma 1$) with a light chain of type kappa. The culture of a biopsy specimen of the diseased skin demonstrated low-speed IgG synthesis. It is concluded that the bone marrow and pathological skin synthesize a paraprotein with the same characteristics as that in the serum.

Microchemical analysis of pathological skin demonstrated an increase in the content of neutral glycoproteins, hyaluronic acid and chondroitin sulphates as well as the presence of heparin and keratan sulphates.

It could not be demonstrated that the serum of this patient contained antibody against connective tissue ground substance or other skin constituents.

Scleromyxoedema (syn. lichen myxoedematosus, papular mucinosis) is a connective tissue disease with characteristic skin manifestations and usually runs a benign course. Thus far, about forty cases have been reported. This disease appears to be frequently associated with paraproteinaemia (Degos *et al.*, 1970; James *et al.*, 1967; Winkelman, 1970; Waldenström, 1971). A monoclonal IgG was demonstrated in nineteen out of a series of twenty-two cases and in nine of them in which the type of light chain of the monoclonal IgG was determined, it proved to belong to the lambda type (Degos *et al.*, 1970). In two other cases IgA and IgM paraproteins were found (Pambor & Höfs, 1968; Bohnstedt & Ehlers, 1967).

In a few cases vague cerebral disturbances have been observed (Donald *et al.*, 1953; Findlay, 1960; Mc.Cuistion & Schoch, 1956). Several authors report a favourable effect of alkeran on the skin lesions and in some cases also on the paraproteinaemia (Degos *et al.*, 1970; Feldman *et al.*, 1969; Winkelman, 1970).

Recently we could observe a patient suffering from this disease with a monoclonal IgG type kappa in the serum, in which the synthesis of the paraprotein could be demonstrated not only in the bone marrow but also in the skin lesions.

CASE REPORT

A 43-year-old male chauffeur-domestic was admitted (November 1969) for redness and thickening of the forehead and pale firm papules on the backs of the hands, forearms, and the extensor surfaces of the feet, which had already persisted for 10 months. In addition, he had complained for the last 6 years of vague headaches and more recently of rapid fatigue and dissatisfaction with his work. Eighteen years ago he had been treated in a hospital for psychoneurotic complaints.

Diagnostic procedures

The thickened red folds on the forehead and the pale papules were characteristic of scleromyxoedema (Fig. 1). The diagnosis was confirmed by the histopathological findings (see below).

General medical and neurological examination disclosed no relevant abnormalities.

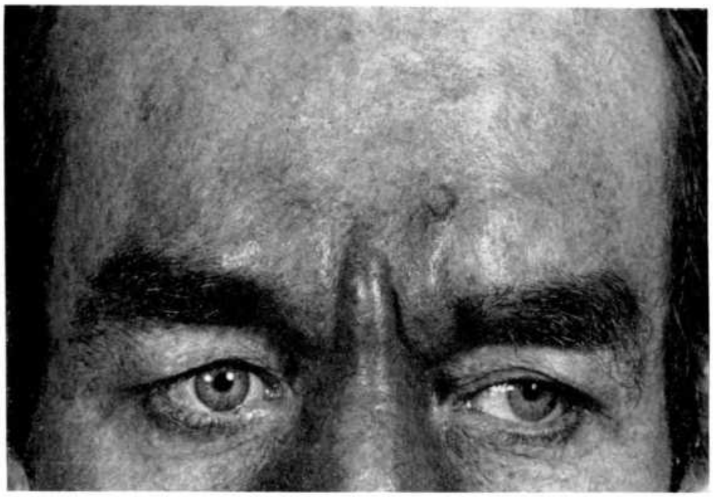

FIGURE 1. Thickening, redness, and folds on forehead.

Laboratory values were within normal limits for the following determinations: erythrocyte sedimentation rate, haemoglobin content (14·6 g%), white blood cell count (5700/mm^3) including differential count, urine analysis, liver and renal function tests, glucose tolerance test, protein-bound iodine 4·9 μg%, total cholesterol (208 mg%), and total serum lipids (755 mg%). X-ray examination of the chest, oesophagus, and skeleton also revealed no abnormalities.

Clinical course and treatment

The patient was treated with alkeran (Melphalan®) in a dose of 4 mg daily, and was seen weekly in the out-patient department. Twenty days later he was re-admitted because of severe, acute mental deterioration with dysarthria and partial loss of consciousness. The skin eruption had not changed. Within a few hours the patient developed a grand mal seizure, followed by a deep persistent coma. The neurological diagnosis was metabolic encephalopathy with cerebral oedema of unknown origin. Extensive internal medical and neurological examinations did not elucidate the nature of the process. Treatment was directed primarily at controlling the cerebral oedema. The patient's condition deteriorated rapidly, his temperature rose to 39·7°C, and he developed bronchopneumonia, probably due to aspiration (sputum culture: *Haemophilus influenzae* and *Staphylococcus aureus*), for which he was treated with penicillin and streptomycin. There was an increasing number of seizures with alternating tonic and clonic periods, and an adequate blood pressure could not be maintained in spite of plasma transfusion. The patient died 2 days after admission.

Autopsy findings: (E.M.J.Corsmit, Department of Pathology)
The autopsy performed immediately after death revealed oedema of both lungs, lobular pneumonia, a few bronchopneumonic foci, and acute tracheitis. Although this picture resembled an influenza virus infection with a secondary bacterial infection, the tissue culture was negative for influenza virus; the bacteriological culture of lung material showed *Staphylococcus aureus*.

There was a slight fibrosis of the myocardium, but without signs of infarctation or atheromatosis.

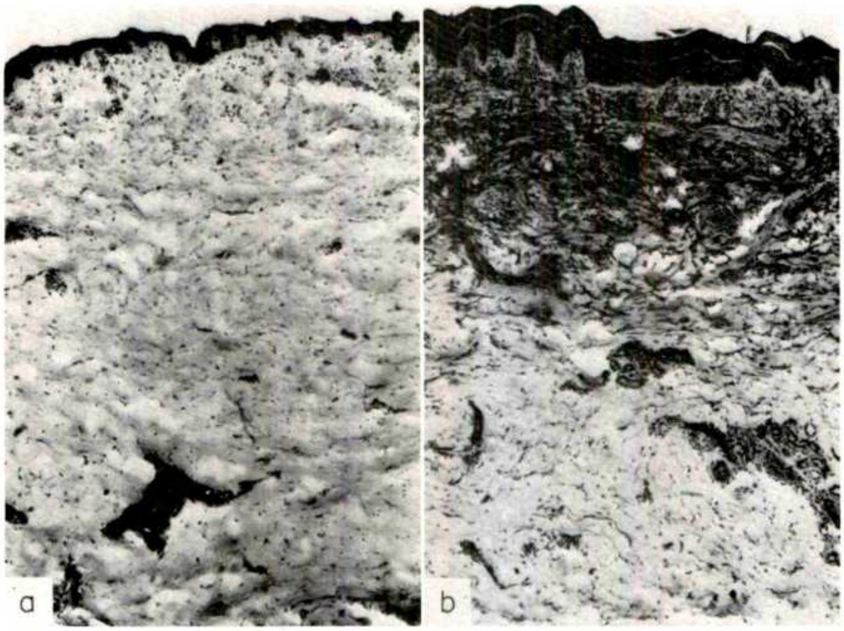

FIGURE 2. (a) Histopathology of the normal skin of patient (× 42). (b) Histopathology of the pathological skin (papule): strong metachromatic reaction in upper layers of corium (Azur A staining, pH 7 (× 42).

The brain showed a marked oedema with some small fresh haemorrhages. Microscopical examination demonstrated that the cerebral oedema could be considered to be of short duration (a few days), the cause of which remained unclear. In addition, there were signs of ependymitis granularis, most pronounced at the bottom of the third ventricle, which must have been present for many years. It is conceivable that the latter had been responsible for the psychoneurotic complaints, which had lasted 6 years.

Specific investigations

Histopathological findings in the skin. The biopsy specimen of a papule showed a marked increase of the connective tissue ground substance in the upper layers of the dermis (stained metachromatically with toluidin blue and azure A), and a slight increase of fibroblasts, as well as loosening of the collagen fibres and fragmentation of the elastic fibres in this area. The number of mast cells was markedly increased. In addition there were lymphocytes scattered through the dermis (Fig. 2).

Analysis of mucopolysaccharides in skin lesions. Quantitative microchemical determination of the acid mucopolysaccharides in the skin lesions was carried out by Dr J.A. Szirmai (Institute for Rheumatism Research, Leiden), according to the method described by Antonopoulos *et al.* (1964).

The material from skin lesions was obtained by punch biopsies. Unfixed cryostat sections (40 nm) were freeze-dried, and the dermis was isolated by microdissection. Apparently normal skin from the same anatomical area was used as control. The results are shown in Table 1. The most relevant findings were: a distinct (2·8-fold) increase of the hyaluronic acid content in the involved skin, a strong (5·5-fold) increase of the chondroitin sulphates and the presence of a small amount of a polysaccharide, part of which could be heparin.

The ratio of chondroitin sulphates to hyaluronic acid was 0·30 (normal ratio 0·15).

The collagen content showed no significant alterations. Determination of serum and urinary mucopolysaccharides was not performed.

TABLE 1. Microchemical analysis of polysaccharide contents in connective tissue of normal and pathological skin

Fractions	Content	Normal skin (2·772 mg) hexosamines			Pathological skin (3·158 mg) hexosamines			
		μg	Percentage of dry substance	Total percentage	μg	Percentage of dry substance	Total percentage	Increase
1% CPC	Neutral glycoproteins	4·7	0·17	53·1	7·8	0·25	32·0	× 1·4
0·3 N NaCl	Hyaluronic acid	3·7	0·13	40·6	11·8	0·37	49·0	× 2·8
1·0 N $MgCl_2$	Chondroitin sulphates	0·7	0·02	6·3	3·3	0·11	15·0	× 5·5
6 N Hcl	Heparin and keratan sulphates	0·2	—	—	1·1	0·03	4·0	—
Total		9·1	0·32	100·0	24·0	0·75	100·0	× 2·3

Immunological studies

Since scleromyxoedema is very frequently associated with paraproteinaemia, an extensive immunological analysis was done.

Blood serum. Paper-electrophoresis showed no important abnormalities (total protein 7·6 g%, albumin 4·9 g%, α_1-globulin 0·2, α_2-globulin 0·5, β-globulin 0·6, γ-globulin 1·4 g%). The agar and the cellulose acetate electrophoresis showed an M component with low speed mobility. Further analysis with the immunoselection method (Rádl, 1970) and subclass typing with immunoelectrophoresis revealed that the paraprotein was IgG_1, type kappa.

No Bence-Jones proteins could be detected in the urine. Quantitative analysis of the serum immunoglobulins by the single radial diffusion technique according to Mancini gave normal values (Table 2).

The serum contained no cryoglobulins or cold agglutinins; the LE cell test, the direct Coombs' test, and the Rose and latex test were also negative. An investigation for the presence of auto-antibodies by immunofluorescence (Dr T. E. W. Feltkamp, Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam) against tissue antigens of thyroid gland (colloid), striated muscle, heart muscle, stomach parietal cells, adrenal cortex, salivary gland, mitochondria, smooth muscle, and nuclei were all negative, except for the finding of a low titre of antibodies against thyroid gland cytoplasm.

TABLE 2. Analysis of immunoglobulins in serum and plasma cells of bone marrow

	M component	Mobility	IgG	IgA	IgM	IgD	K/λ ratio
Cellulose acetate electrophoresis	γ region	Low speed					
Immunoelectrophoresis and immunoselection	IgG_1, type K	Low speed					
Immunoglobulins contents of serum* (mg %)			1197 (664–1401)	119 (103–404)	94 (55–141)		
Immunoglobulin containing plasma cells in bone marrow† (rel. %)			78 (51)	11 (37)	8 (12)	3 (4)	4·6 (2·2)

* According to the Mancini technique. Normal range between brackets (Stoop *et al.*, 1969).

† Direct immunofluorescence technique. Normal mean values between brackets (Hijmans *et al.*, 1971).

Bone marrow. Morphological examination showed 2% plasma cells and a slight increase of mast cells locally.

Immunofluorescence examination of a fixed bone marrow sample (Miss H. R. E. Schuit, Institute for Rheumatism Research, University Hospital, Leiden) showed an increased percentage of IgG-positive cells, a slight decrease of the IgA- and IgM-positive cells, and a normal percentage of IgD-positive cells.

The kappa to lambda ratio of all immunoglobulin-containing cells was higher than normal (Table 2).

Skin. Immunofluorescence examination of the pathological skin with transmitted light, revealed no immunoglobulin deposits and no Ig-synthesizing cells. Indirect immunofluorescence investigations after incubation of pathological skin with the patient's serum also revealed no abnormalities.

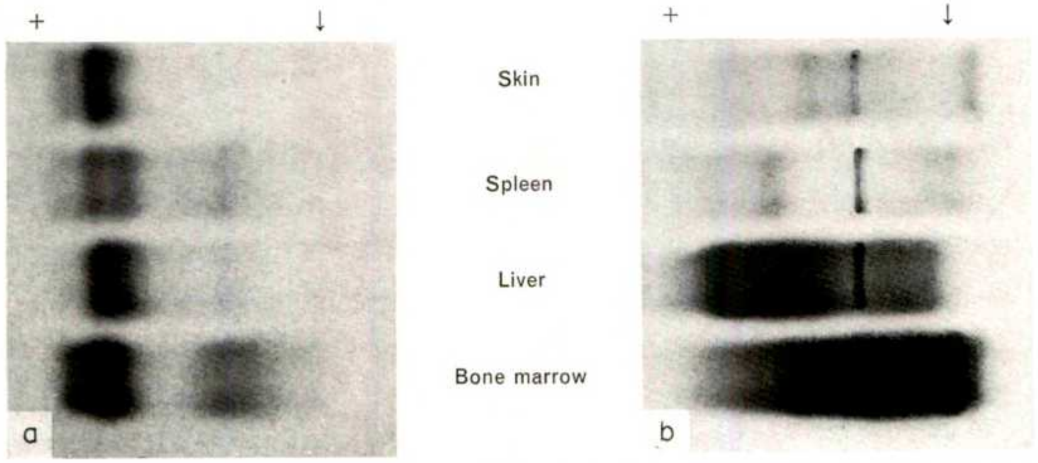

FIGURE 3. (a) Cellulose acetate membrane with the electropherographs of culture fluids. (b) Autoradiographs showing a homogeneous band in γ-globulin region (↓) in culture fluid of skin and bone marrow.

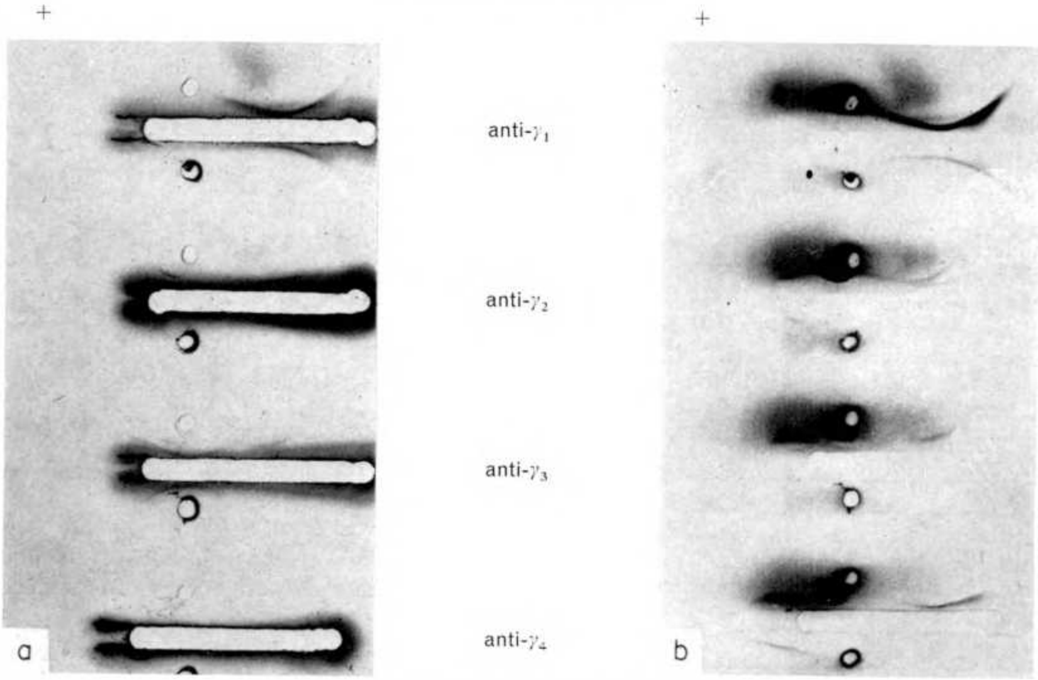

FIGURE 4. Subclass analysis of IgG synthesized by the bone marrow. The wells above the troughs are filled with culture fluid of the bone marrow. (a) Immunoelectrophoretic patterns developed with anti-γ_1, γ_2, γ_3, and γ_4 sera. (b) Autoradiograph showing a low-speed IgG paraprotein in bone marrow culture fluid. Note that the subclass antisera are not completely monospecific. For example, with the sensitive technique of autoradiography two IgG lines are visible in the autoradiograph but only one in the immunoelectropherograph developed with anti-γ_2 serum; furthermore, in the autoradiograph of the immunoelectrophoretic pattern developed with anti-γ_4 serum a γ_1 line is visible.

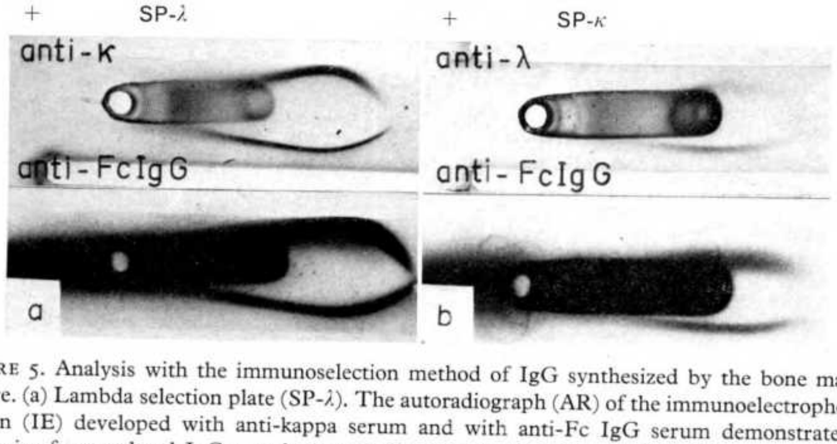

FIGURE 5. Analysis with the immunoselection method of IgG synthesized by the bone marrow culture. (a) Lambda selection plate (SP-λ). The autoradiograph (AR) of the immunoelectrophoretic pattern (IE) developed with anti-kappa serum and with anti-Fc IgG serum demonstrates the synthesis of monoclonal IgG type kappa. (b) Kappa selection plate (SP-K). The autoradiograph of the immunoelectrophoretic pattern developed with anti-lambda serum and anti-Fc IgG serum demonstrate the production of IgG type lambda lacking the characteristics of a paraprotein.

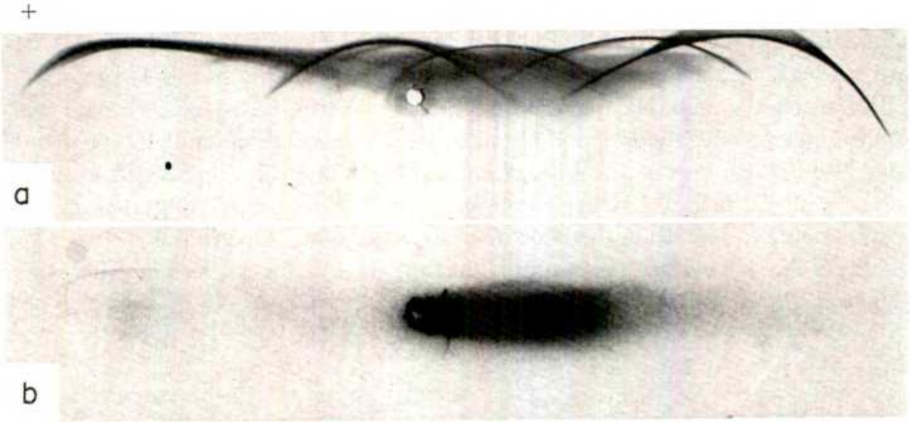

FIGURE 6. (a) Immunoelectrophoretic pattern of carrier serum and culture fluid of pathological skin developed with horse anti human serum. (b) Autoradiograph showing labelling of low-speed IgG line.

Immunoglobulin synthesis in vitro. For the assay of immunoglobulin synthesis *in vitro* a bone marrow sample, specimens of skin lesions, and samples of spleen and liver tissue (both post-mortem) were cultured according to Hochwald, Thorbecke & Asofsky (1961) as described in detail by van Furth, Schuit & Hijmans (1966b).

Briefly, this method is as follows. Bone marrow flakes or fragments of minced tissue are incubated for 48 h in a modified Eagle's medium containing ^{14}C-lysine (310 μC/mmole) and ^{14}C isoleucine (312 μC/mmole) with high specific activity. The culture fluid was analysed by cellulose acetate electrophoresis, immunoelectrophoresis, and the immunoselection method (Rádl *et al.*, 1972) using specific antisera against heavy or light chains. In the immunoelectrophoretic analysis a carrier serum was used to obtain well-defined and reproducible precipitation lines. The *de novo* synthesized immunoglobulins were detected and identified by autoradiography of the electrophoretic pattern of the culture fluids. The results are shown in Figs. 3–6 and Table 3.

The autoradiograph of the cellulose acetate membrane shows a homogeneous band at the cathodal site in the culture fluid of the skin and bone marrow, whereas this band was not found in the liver and spleen cultures (Fig. 3). The autoradiograph of the immunoelectrophoretic pattern of the bone

TABLE 3. Immunoglobulin synthesis in various tissues

Origin	Cellulose acetate electrophoresis		Synthesis of immunoglobulins*			Subclass/type
	M component†	Mobility†	IgG	IgA	IgM	M component‡
Bone marrow	γ-region	Low speed	+++	++	+	Ig G_1-K
Skin lesions	γ-region	Low speed	+	–	–	
Skin lesions§	γ-region	Low speed	(+)	(+)	–	
Spleen§	negative		++	+	–	
Liver§	negative		(+)	–	–	

* The intensity of the autoradiographic lines is classified from: – = negative, (+) = just visible, + = clearly visible, to a maximum of ++++.

† Results autoradiography of cellulose acetate electrophoresis pattern.

‡ Result autoradiography of immunoselection plate and immunoelectrophoresis.

§ Post-mortem and infected.

marrow culture developed with a horse antihuman serum (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam) showed a low-speed paraprotein in the γ region.

The analysis with subclass specific antisera (Nordic Pharmaceuticals and Diagnostics, Tilburg, Holland) demonstrated autoradiographically that the paraprotein consisted of γ_1 molecules (Fig. 4).

The results obtained with the immunoselection method, in which an antilight chain antiserum is incorporated into the agar (e.g. antilambda serum) and the immunoelectrophoretic pattern developed with the other antilight chain serum (e.g. antikappa serum) and with anti-Fc IgG serum, showed that the IgG paraprotein synthesized in the bone marrow was of type kappa (Fig. 5).

The analysis of the skin culture demonstrated that only IgG with low-speed mobility was produced *in vitro* (Fig. 6).

DISCUSSION

Until recently, little was known about the site of synthesis of the monoclonal protein in scleromyxoedema. In a previous paper, Suurmond & van Furth (1969) described a patient with scleromyxoedema associated with an IgG type lambda paraproteinemia, in which the paraprotein proved to be synthesized in the bone marrow. Immunofluorescence studies with transmitted light failed to demonstrate the presence of immunoglobulin in the skin lesions. Similar negative results have been reported by James *et al.* (1967), Piper, Hardmeier & Schäfer (1967), Fowkles, Blaylock & Mullinax (1967), Delacretaz *et al.* (1970) and Degos *et al.* (1970). Positive results have been claimed only by McCarthy *et al.* (1964), who found a marked fluorescence (immunoglobulin deposits ?) of the corium treated with fluorescein-labelled rabbit antihuman gammaglobulin.

The present case shows two unique features: first, the occurrence of a monoclonal IgG of type kappa in the serum and secondly, the analysis of the culture fluid by cellulose acetate electrophoresis, immunoelectrophoresis, and the immunoselection method indicated that the bone marrow synthesized monoclonal IgG with the same electrophoretic mobility (low speed, subclass γ_1) and the same type of light chain (type kappa) as were found in the serum, and that the tissue culture of the diseased skin also produced monoclonal IgG with low-speed mobility. The type of light chain of the monoclonal IgG produced in the skin could not be identified with certainty, because of the relatively small amount of synthesized radioactive protein. To the best of our knowledge, this is the first time that the synthesis of a paraprotein in the skin has been demonstrated.

In other cases of paraproteinaemia, the synthesis *in vitro* of monoclonal immunoglobulins in bone marrow samples of patients suffering from multiple myeloma, Waldenström's disease, chronic lymphatic leukemia and the so-called benign monoclonal gammopathy (Waldenström) have been reported (van Furth *et al.*, 1966a, 1967).

In all instances the class and type of monoclonal immunoglobulin(s) synthesized *in vitro* were identical to those of the paraprotein(s) in the serum. In these cases the results of the immunofluorescence studies of bone marrow samples were in accordance with the results of the culture studies, showing a distinct increase (>70%) or a predominance (about 99%) of plasma cells containing one class and type of light chains (van Furth *et al.*, 1966a, 1967).

The lymphoid cells which synthesize the monoclonal immunoglobulins in the skin are probably cells originating in lymphoid organs and which have migrated to the skin via the blood vessels. In all likelihood the number of immunoglobulin-synthesizing cells is rather low since immunofluorescence analysis with transmitted light showed no positive cells. Furthermore, the investigation was previously hampered because the sensitivity of the only method formerly available, was inadequate for detection of small amounts of immunoglobulin in lymphoid cells.

Paraproteins have been found to have antibody activity against a great variety of antigens. In the present study no evidence was obtained indicating that the paraprotein in the serum of this patient had antibody activity against connective tissue ground substance or any other constituents of the skin, since indirect immunofluorescence studies with the patient's serum were negative.

It is still not clear what (immunological) role the paraproteins play in this disease.

It remains possible that the paraprotein has antibody activity against an unknown antigen specific for this disease. Then the question arises whether these paraproteins exert a certain homeostatic mechanism and in this way control the progress of the disease. Another possibility is that the paraprotein has nothing to do with the disease, but this might be in contradiction with the synthesis of the paraprotein in the skin. Further investigations are needed to elucidate these problems.

ACKNOWLEDGMENTS

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Studies on a patient with leucocytoclastic vasculitis 'pyoderma gangrenosum' and paraproteinaemia

DOROTHY M.THOMPSON, ROBERT A.MAIN, *J.SWANSON BECK, AND FRANK ALBERT-RECHT

Department of Dermatology, Aberdeen Royal Infirmary, and Departments of Pathology and Chemical Pathology, University of Aberdeen

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SUMMARY

A patient with leucocytoclastic vasculitis, pyoderma gangrenosum, chronic pyelonephritis and a paraprotein of IgA type is described. The vasculitis responded favourably to dapsone therapy, while the pyoderma gangrenosum improved only when steroids were also given. During an exacerbation following withdrawal of dapsone, the serum IgG level fell early, but the β_{1C} globulin level remained normal. Immunofluorescence studies on a vasculitic lesion showed granular deposits of IgG and β_{1C} in the walls of the blood vessels and a more extensive exudate of fibrin. Skin testing with bacterial antigens and patch testing to potassium iodide produced lesions which clinically, histologically and immunologically resembled the spontaneous vasculitic lesion.

The pathological term leucocytoclastic vasculitis embraces the clinical conditions of pentasymptome of Gougerot and erythema elevatum diutinum. The overlap in the clinical features of these disorders is considerable and a remarkable improvement with dapsone therapy has been described in both (Vollum, 1968; Wells, 1969; Cream, Levene & Calnan, 1971). The distinction between the two would appear to be artificial. The aetiology of leucocytoclastic vasculitis may be a naturally occurring Arthus reaction (Cochrane & Weigle, 1968; Stringa *et al.*, 1967).

In the light of our present ignorance 'pyoderma gangrenosum' remains only a clinical entity and describes a chronic indolent ulcer with undermined edges and with a tendency to heal at the centre while still extending at its periphery. An association with a number of immunopathological disorders has been noted.

CASE REPORT

The earlier period of this patient's disease was described in 1966 when she was presented to the North British Dermatological Society (Main, 1967): this appeared to be the first description of the dramatic response of leucocytoclastic vasculitis to dapsone.

This woman was first seen in 1963, when she was 51, with recurrent crops of lesions that took a variety of forms. Urticarial, papular, vesicular, bullous and plaque-like lesions, usually with a haemorrhagic component, had been present. Arcuate lesions were sometimes noted (Figs. 1 and 2). The distribution of the lesions was often symmetrical and they appeared most commonly on the extensor aspects of elbows, knees, legs, buttocks and shoulders. Other areas, including the face, were occasionally involved. Over the next 2 years she continued to produce crops of lesions. The outbreaks were associated with ankle swelling and with occasional transient joint pains, but with no other constitutional symptoms. She had recurrent urinary infections, and a pure growth of *Escherichia coli* was usually found on culture of her urine. There appeared to be no chronological relationship between the exacerbations of her vasculitis and the urinary infections. Repeated courses of antibiotics, including a 3 month course of three antibiotics given weekly in rotation, had no effect on her vasculitis.

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* Now Professor of Pathology, University of Dundee.

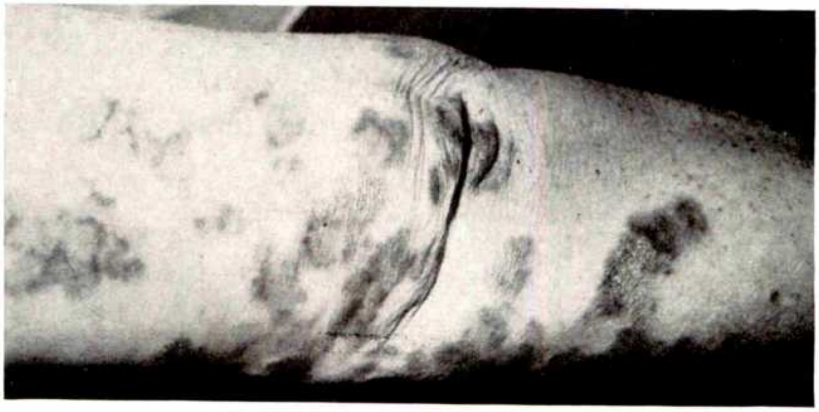

FIGURE 1. Leucocytoclastic vasculitis: erythematous and haemorrhagic lesions with arcuate configuration on extensor aspect of elbow.

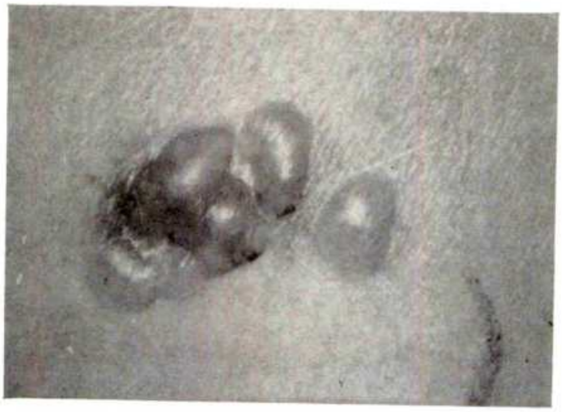

FIGURE 2. Early potassium iodide induced lesion.

In August 1965, she was treated with 30 mg prednisone daily. The vasculitis did not improve, and when the dose was increased over a short period, the process worsened. This treatment was stopped after 3 months.

In June 1966, her vasculitis was severe and widespread. Since the appearance and distribution of the rash had a resemblance to dermatitis herpetiformis, she was treated with dapsone, 50 mg daily. Within 7 days, the eruption had cleared, leaving behind only staining. Since then, her vasculitis has been kept under control with dapsone. Her usual requirement is 25 mg daily, but at times it has been as low as 15 mg daily. On six occasions the drug treatment has been stopped. On each occasion the vasculitis recurred within 48 h. After resumption of therapy, it resolved equally quickly. On one occasion, a placebo was substituted for dapsone, and again a relapse occurred.

Past history

As a child she had rheumatic fever.

Investigations

Normal or negative results: Hb, WBC, platelets, blood film; prothrombin time; clotting time; cephalin cholesterol time; clot reaction; thrombin clotting time; fibrinogen level; euglobulin lysis time; cold agglutinins; cryoglobulins; serum vitamin B_{12}; serum folate; serum iron; TIBC, urea and electrolytes; liver function tests; SGOT, SGPT; serum cholesterol; faecal occult bloods; BSP retention; glucose tolerance test; ECG; X-ray chest, abdomen, IVP; ASO titre; Brucella agglutination tests; Rose Waaler; latex fixation tests; WR and VDRL; ANF and auto-antibodies (repeatedly).

Abnormal investigations: ESR—40 mm in first hour. MSSU—growth of *E. coli* more than 100,000 organisms/ml. WBC excretion rate—487,000 WBC/h. Serum proteins and electrophoresis—slight increase in β globulin.

Course

In October 1967, she developed serpiginous ulcers on her right thigh and arm. These ulcers extended slowly, had overhanging violaceous edges and fulfilled our diagnostic criteria for pyoderma gangrenosum. A biopsy was taken from an active, spreading, infiltrated edge.

This lesion did not respond to dapsone even when the daily dose was increased to 100 mg. When she was given 40 mg prednisone in addition, the ulcers healed leaving thin papery scars. The dose of prednisone was gradually reduced to 5 mg daily. On two subsequent occasions she developed similar ulcers. These healed when the daily dose of prednisone was increased to 20 mg. Steroid treatment was stopped in December 1970 and, to date, the pyoderma gangrenosum has not recurred.

In December 1970, an abnormally dense compact band was noted for the first time on electrophoresis of serum proteins. There were no abnormalities suggestive of myelomatosis, reticulosis, or ulcerative colitis. Further investigations were carried out in the light of these findings.

Serum proteins. Albumin 4·3 g: α globulin 0·3 g: α_2 globulin 0·7 g: β globulin 1·0 g: γ globulin 0·5 g: abnormal protein 0·7 g: (usual values: albumin 4·00–4·90 g: α globulin 0·08–0·22 g: α_2 globulin 0·43–0·57 g: β globulin 0·37–0·73 g: γ globulin 0·95–1·23 g.). Abnormal band between β and γ globulins. Immunoglobulins. IgG 1660 mg (normal 700–1800 mg), IgA$>$1100 mg (normal 90–650 mg), IgM 84 mg (normal 60–280 mg).

Sternal marrow (smears and sections)—'moderate plasmacytosis with no abnormal plasma cells. No evidence of myelomatosis'. Bence Jones protein in urine absent (after concentration of urine). Serum calcium, phosphate, alkaline phosphatase normal. X-radiographic skeletal survey negative.

Biopsy of pyodermatous lesion

Throughout the dermis and subcutis but with maximal concentration about the blood vessels, there is an infiltrate of acute inflammatory cells. These cells, which exhibit marked leucocytoclasis, are mainly polymorphonuclear neutrophils but abundant eosinophils are present. The blood vessels exhibit mural fibrinoid change.

Special investigations

Immunoglobulins and complement level. Dapsone was withdrawn and an exacerbation of vasculitis began within 48 h. Before and during the exacerbation, serial estimations of immunoglobulins and serum complement were carried out. Immunoglobulins were estimated in triplicate on one plate using radial immunodiffusion techniques. Serum complement was estimated on three specimens on one plate using Hyland immunodiffusion plates. The results of these investigations are shown in Fig. 3.

Reactions to bacterial antigens. 0·1 ml of Bencard's bacterial antigenic suspensions, containing 5×10^8 organisms/ml, F1-5, *Staphylococcus aureus*, *S. albus*, *Escherichia coli* and control were injected intradermally into the patient's back.

These tests were repeated on two further occasions while she was taking dapsone, 25 mg daily.

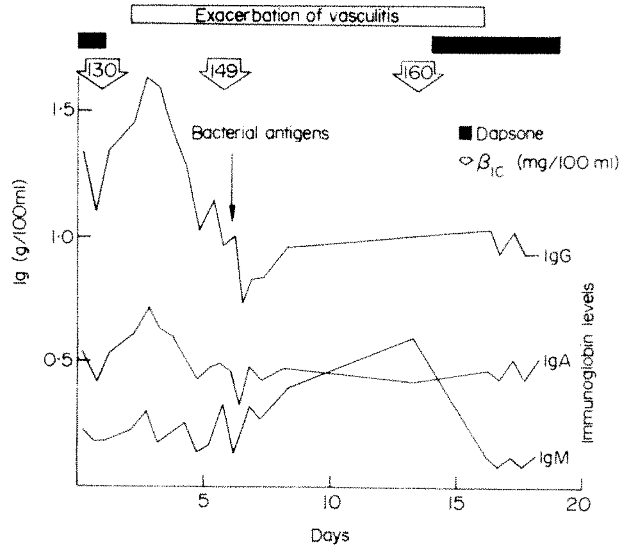

FIGURE 3. Serum immunoglobulin and β_{1C} levels during exacerbation of vasculitis.

An immediate erythema was produced with all the bacterial antigens and with the control. A second response was noted at sites of injection of all the antigens, but not with the control. This reaction began at 2 h and increased gradually until 4–5 h. The lesions produced were erythematous nodules with a haemorrhagic centre. The largest and most persistent lesions occurred with *E. coli* and F5 group which contains *E. coli*. The same response was obtained when the tests were repeated. A biopsy was taken from a nodular haemorrhagic lesion present at 6 h at the site of injection of *E. coli* antigen.

Potassium iodide patch tests. Patch testing with 25% potassium iodide in yellow soft paraffin produced a vesicular lesion after 8 h, and a haemorrhagic lesion after 24 h (Figs. 2 and 4). Lower concentrations of potassium iodide and yellow soft paraffin as control gave negative results. Biopsies were taken of a vesicular lesion 4 h old, present at 12 h, and a haemorrhagic and vesicular lesion 25 h old, present at 48 h, at sites of application of 25% potassium iodide patch test.

A biopsy was also taken from a spontaneously arising nodular haemorrhagic lesion approximately 24 h old.

One biopsy, that from the 4-h-old potassium iodide induced lesion, was treated with immunofluorescence technique alone; the other biopsies were bisected. Half was fixed in formalin and paraffin sections were stained with haematoxylin and eosin. The other half was snap-frozen and 5 μm cryostat sections were stained for IgG, IgA, IgM, β_{1C} and fibrin with the indirect immunofluorescence technique, using commercial rabbit antisera (Behringwerke) whose specificity in this technique has been verified, according to the criteria of Beck & Currie (1967), in previous investigations (Simpson & Beck, 1971—unpublished) (Figs. 8 and 9).

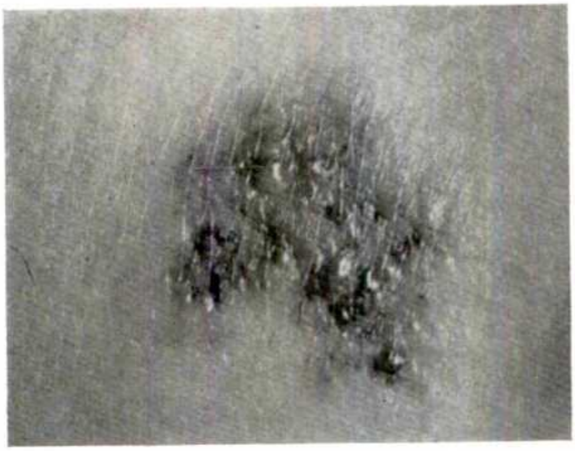

FIGURE 4. Late haemorrhagic and vesicular lesion induced by potassium iodide.

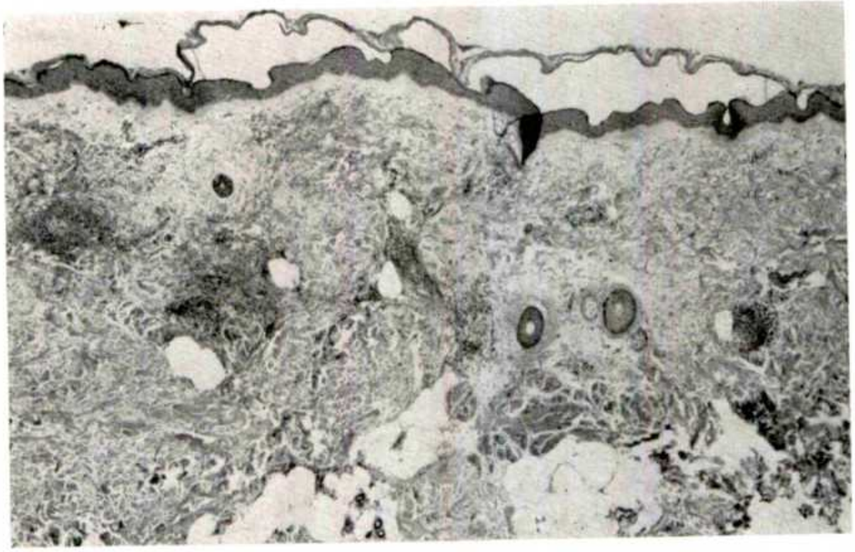

FIGURE 5. Biopsy of spontaneous lesion on upper arm showing focal perivascular and peri-appendicular inflammation in dermis (H & E, × 36).

Biopsy reports

(1) *Spontaneous lesion.* There is a focal dermal infiltrate of polymorphonuclear leucocytes around the smaller dermal vessels. Leucocytoclasis is present. The vessels show fibrinoid necrosis (Figs. 6 and 7).

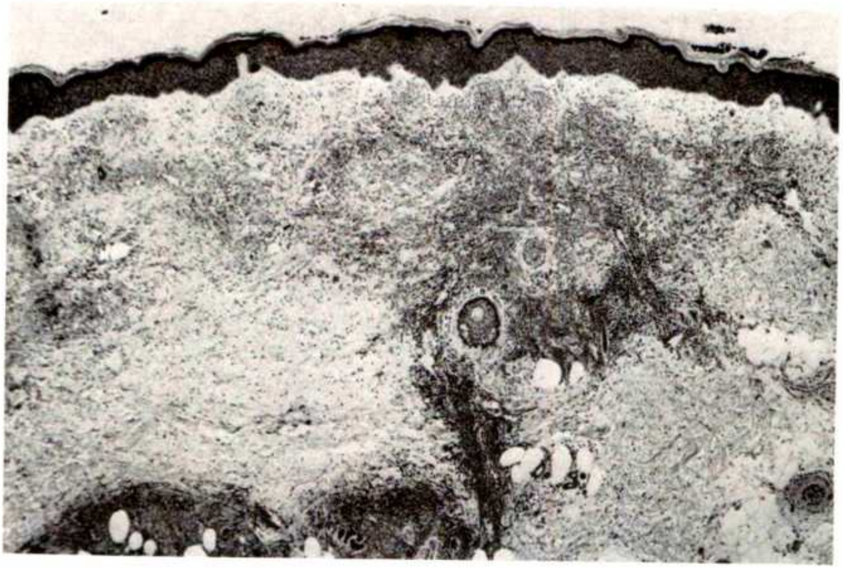

FIGURE 6. High power view of biopsy of spontaneous lesion in dermis showing that inflammatory cells are mainly polymorphonuclear leucocytes many of which show leucocytoclasis: there is disorganization of several blood vessels (H & E, × 240).

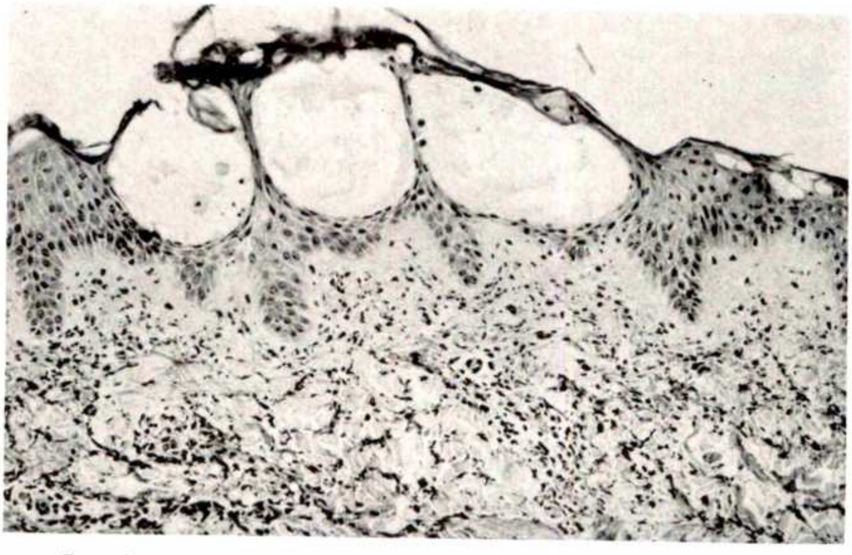

FIGURE 7. Potassium iodide induced lesion on back. The epidermis shows marked spongiosis and vesiculation. The dermis shows focal polymorphonuclear leucocyte infiltration and leucocytoclasis (H & E, × 240).

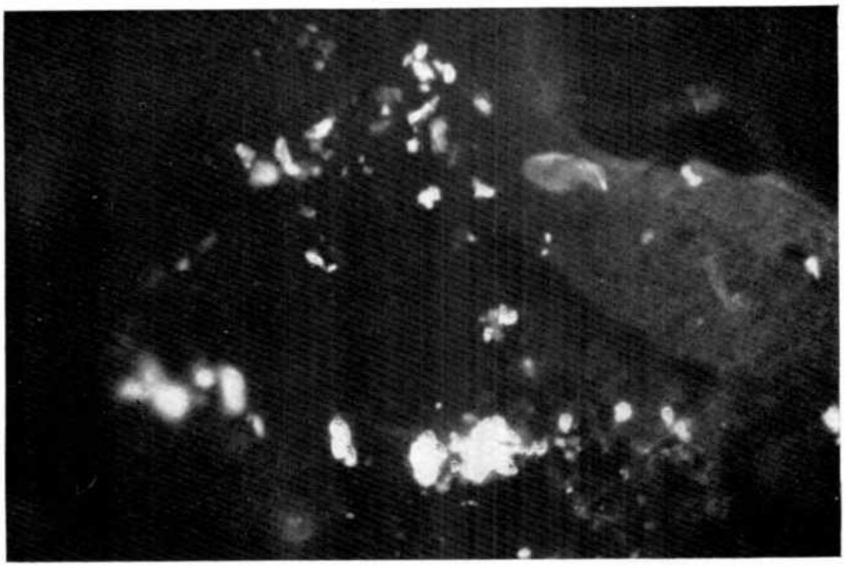

FIGURE 8. Immunofluorescence staining of spontaneous leucocytoclastic vasculitis lesion with rabbit antihuman IgG serum and fluorescein conjugated goat antirabbit Ig serum. There are granular deposits of specifically stained material in the inflamed tissue adjacent to a venule (lower left) (× 750).

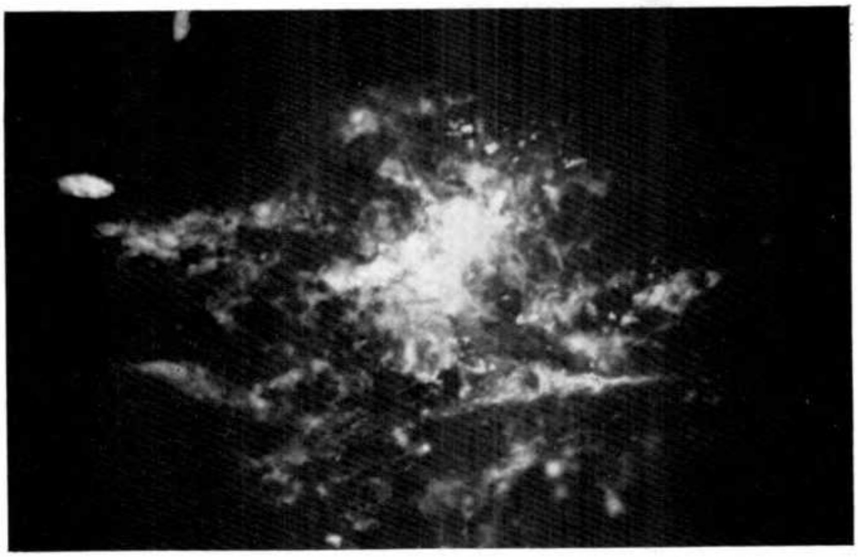

FIGURE 9. Immunofluorescence staining of spontaneous leucocytoclastic vasculitis lesion with rabbit antihuman fibrinogen serum and fluorescein conjugated antirabbit Ig serum to demonstrate fibrin in exudate. There is intense staining in the wall of a damaged vessel with diffuse staining within the dermal collagen (× 400).

(2) *Bacterial antigen injection site.* There is a marked peri-appendageal and perivascular acute inflammatory infiltrate in the dermis and subcutis. The infiltrate is composed mainly of polymorphonuclear neutrophils but eosinophils are very conspicuous. Leucocytoclasis is also present. Some of the vessel walls have been destroyed by the infiltrate and some show fibrinoid necrosis.

(3) *Potassium iodide reaction.* There is a leucocytoclastic vasculitis with fibrinoid necrosis and an infiltrate of polymorphonuclear leucocytes which exhibit leucocytoclasis. The epidermis shows marked spongiosis and a sparse infiltrate of lymphocytes and polymorphs (Fig. 7).

Immunofluorescence studies

In the early potassium iodide induced vesicular lesion, no specific staining of immunoglobulins, β_{1C} globulins, or fibrin was seen with the immunofluorescence techniques. By contrast, the spontaneous lesion, the late potassium iodide induced lesion, and the bacterial antigen induced lesions showed specific immunofluorescence staining in a similar distribution. IgG and β_{1C} globulin were localized as granular deposits (approximately 1–5 μm diameter) predominantly in the walls of the small blood vessels (mainly arterioles) and to a lesser extent in peri-adventitial area of dermis. Fibrin was present not only in the walls of affected vessels but also in the rather extensive inflammatory exudate in the dermis. Neither IgA nor IgM was localized in the lesions.

DISCUSSION

The spontaneous lesion, the *E. coli* antigen induced lesion and the potassium iodide induced lesion all showed the histological appearances of a leucocytoclastic vasculitis, and immunofluorescence studies showed in each the presence of IgG/β_{1C} in the blood vessel walls. These findings are compatible with an Arthus reaction. The timing of the bacterial antigen induced lesion, beginning at 2 h and reaching a maximum at 4–5 h is also compatible with an Arthus reaction. In the two patients described by Cream *et al.* (1971) the histological reaction to streptococcal antigen was a leucocytoclastic vasculitis but the timing was more suggestive of a delayed reaction.

Immunoglobulins have been estimated in patients with vasculitis (Copeman, 1970) but it is possible to regard these only as random observations. In this patient immunoglobulins were followed during the course of the exacerbation. The withdrawal of dapsone was followed by an initial rise in IgG and then by a marked fall. Whether the quantitative change in IgG serum concentration are fully explicable by its incorporation into vessel wall deposits is uncertain.

In the aetiology of dermatitis herpetiformis, subcorneal pustular dermatosis and leucocytic vasculitis, those disorders in which a remarkable improvement with dapsone therapy is found, immune complexes have been implicated (Van der Meer, 1969; Krogh & Tönder, 1970; Stringa *et al.*, 1967). A polymorphonuclear infiltrate is a conspicuous feature of each disease. The mode of action of dapsone is unknown but it may act at some stage in the train of events between the formulation of immune complexes and the tissue damage caused by release of lysosomes from polymorphs.

Whether pyoderma gangrenosum falls into the spectrum of leucocytoclastic vasculitis is uncertain. In our patient the histology of the active spreading edge of a pyodermatous lesion was similar to the vasculitic lesion. The clinical appearances, behaviour and response to treatment, were, however, quite different.

This patient had an IgA paraprotein band on immunoelectrophoresis. Fifteen patients have been reported with pyoderma gangrenosum in association with a monoclonal immunoglobulin. In eleven patients, the abnormal protein was an IgA (Duperrat, Gougual & Galy, 1962; Thivolet *et al.*, 1964; Hermier-Abgrall, 1965; van der Sluis, 1966; Jablonska, Stachov & Dabrowska, 1967; Schröpl, 1967), in two an IgG (Degos *et al.*, 1966; Imhof *et al.*, 1969) and in one an IgM (Cream, 1971). An association between erythema elevatum diutinum and myeloma has been reported (Duperrat & Rappaport, 1958) and between 'necrotizing' vasculitis and myeloma (Mitchell Sams, Harville & Winkelmann, 1968). The vasculitis in this patient also became worse with steroid therapy.

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Immunological associations of the halo naevus with cutaneous malignant melanoma*

P.W.M.COPEMAN, M.G.LEWIS†, T.M.PHILLIPS† AND P.G.ELLIOTT

Westminster Hospital, London, S.W.1

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SUMMARY

Antibodies against the cytoplasm of malignant melanoma cells have been found formerly to be circulating in our patients with primary malignant melanoma before metastases had developed. We have found now this same antibody in fifteen patients with resolving halo naevi. It disappears once resolution of the naevus is complete or after a solitary active halo naevus has been excised.

However, cytoplasmic antibody has not been detected in the serum of any other patients with cutaneous pigmentary disorders: including fifty-six with various pigmented tumours or naevi, many of them referred to us as suspected malignant melanoma; forty-seven with vitiligo unassociated with halo naevi; and six with benign juvenile melanoma.

We postulate that:

(1) Halo naevus represents an accelerated and successful rejection phase of a pigmented mole developing malignant change.

(2) There are at least two aetiological types of vitiligo, the common variety and another one associated specifically with halo naevus, perhaps initiated by the same immunological stimulus or its sequelae.

(3) Juvenile (benign) melanoma is not related immunologically to malignant melanoma.

During recent studies into immunological host reactions in patients with cutaneous malignant melanoma (Lewis, 1967; Lewis & Kiryabwire, 1968), circulating tumour-specific antibodies were found in those patients with early or localized tumours, but not in those with disseminated melanoma (Lewis *et al.*, 1969). These tumour antibodies were demonstrated by complement-dependent cytotoxicity tests (Pulvertaft, 1959; Lewis, 1967), immunofluorescence microscopy (Morton *et al.*, 1968; Muna, Marcus & Smart, 1969; Lewis *et al.*, 1969; Phillips & Lewis, 1970), radioactive isotope uptake studies (Ikonopisov *et al.*, 1970), and have been confirmed recently by a variety of immunodiffusion and immuno-electrophoretic techniques (Kohn, 1968; Lewis *et al.*, 1971).

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† Present address: Faculty of Medicine, Memorial University of Newfoundland, St John's, Newfoundland, Canada.

Requests for reprints: Dr P.W.M.Copeman, Department of Dermatology, Westminster Hospital, London, S.W.1.

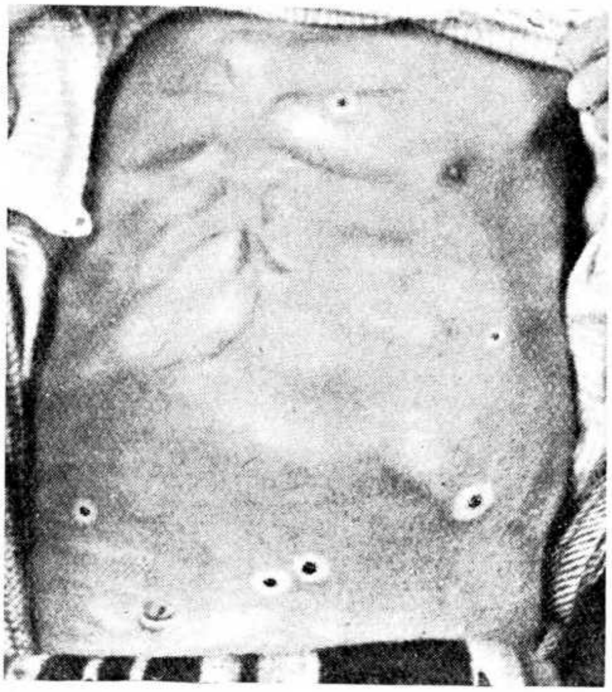

FIGURE 1. Rolleston's (1910) patient with multiple halo naevi.

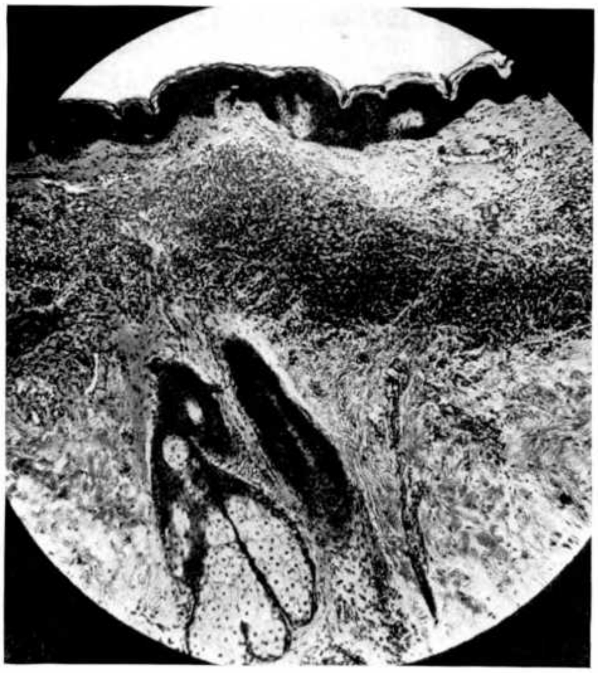

FIGURE 2. Sutton's (1916) patient. Histopathology of halo naevus. A massive lymphocytic infiltrate surrounds the naevus cells.

Immunofluorescent microscopical methods demonstrated two distinct types of antigen in the melanoma cell. They were situated in:

(1) the *cytoplasm* of snap-frozen malignant melanoma cell smear preparations and short-term cultured malignant melanoma cells. The cell cytoplasm stained positively with a fluorescein labelled antiserum after treatment with positive serum from the same or another patient;

(2) the *membrane* (Moller & Moller, 1964; Klein *et al.*, 1966) of living malignant melanoma cells. Significant titres however were seen in the sera only of individual patients against their own tumour cells (Phillips, 1971; Phillips & Lewis, 1970).

This apparent common antigenic component in the cytoplasm of melanoma cells was investigated further with a view to assessing whether it could be exploited for diagnosis and prognosis in patients with malignant melanoma or other pigmented tumours. Patients were investigated with various pigmented skin lesions, both neoplastic and non-neoplastic, many of whom later had histopathological confirmation of the nature of their lesions.

The halo naevus is a distinctive self-healing melanocytic mole. Its pathogenesis and significance has been speculated upon (Hyde, 1906; Rolleston, 1910—Fig. 1; Sutton, 1916—Fig. 2; Feldman & Lashinsky, 1936; Kopf, Morill & Silberberg, 1965; Wayte & Helwig, 1968); the naevus for long having being remarkable for spontaneously regressing while at the same time developing a depigmented halo in the otherwise normal surrounding skin.

The sera from these patients with halo naevi were the only ones other than those from patients with early or localized malignant melanoma to show a positive reaction against the cytoplasmic antigen of malignant melanoma cells. This paper, an extension of our preliminary communication (Lewis & Copeman, 1972), presents these findings and discusses their possible significance in relation to the immunology of melanoma and the pathogenesis of the halo naevus.

MATERIALS AND METHODS

Antigenic test substrate (malignant melanoma cells)

(1) *Smears.* A single cell suspension was prepared from fresh, surgically removed tumour obtained from patients with malignant melanoma (Lewis *et al.*, 1969). Smears on microscope slides were made (in a similar manner to blood smears) from the suspension, which had previously been adjusted to 1×10^6 cells/ml. These smears were quickly dried at 37°C, snap-frozen in liquid nitrogen/isopentane (Nairn, 1969; Phillips & Lewis, 1970), and then stored at −136°C in vapour-phase liquid nitrogen.

(2) *Tissue culture.* Malignant melanoma cells were cultured in short-term chambers as described previously (Lewis, 1967). After 5 days the coverslips were removed. The cells sticking to the coverslips were snap-frozen in liquid nitrogen/isopentane. The indirect immunofluorescent staining technique was then applied as described below.

(i) *Patients' sera*: Blood was taken from patients with a variety of skin lesions who had had histopathological confirmation of the diagnosis, although the diagnoses were not made available until after the results of the immunofluorescence test had been recorded. Samples of sera were dispensed into small aliquots, and were stored at −136°C.

(ii) *Fluorescein labelled antisera.* The conjugate was prepared by injecting sephadex G.200 pure IgG into goats (Ironside, 1968). The IgG of the resulting antiserum was removed by DEAE cellulose and conjugated to chromatographically pure isomer 1 fluorescein isothiocyanate by the dialysis method of Clark & Shepard (1963). The conjugation procedure was controlled so that the resulting F:P ratio lay between 2:1 and 4:1 (White, 1970).

After conjugation, the purification of the labelled antiserum was carried out by bio-absorption chromatography (Stephen, Gallop & Smith, 1966). This process made the conjugate both chromatographically and immunologically pure. Strict control was maintained to ensure that the correct working

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dilution was achieved by the checkerboard titration method against a positive of known typed activity (Beutner, Holborow & Johnson, 1967; Feltkamp, 1970).

(iii) *Immunofluorescent tracing of antibodies.* Serum antibodies were detected against the cytoplasm of the snap-frozen malignant melanoma cells by indirect immunofluorescence microscopy (Holborow & Johnson, 1967; Phillips & Lewis, 1970). The test smears were washed for 30 min in phosphate buffered saline (PBS) 0·01 M PO_4 pH 7·2, to remove all of the protective isopentane. After excess buffer had been removed from around the smears, they were air dried. All sera were diluted 1:4 with PBS. One drop of each serum was applied to each of the five smears on the same microscope slide in the standard order as follows:

Smear 1. Known positive malignant melanoma serum (the same serum being used throughout the test batch).
Smear 2. Known negative malignant melanoma serum (the same serum being used throughout the test batch).
Smear 3. Conjugate control using PBS.
Smears 4–6. Test sera.

The smears were left in a moist chamber at room temperature for 20 min. They were rinsed in a gentle jet of PBS and washed in a PBS bath for 30 min to remove all unreacted serum. After removal from the bath, all excess moisture was gently mopped with a clean cloth from the smears. One drop of goat antihuman gammaglobulin, conjugated to isomer 1 FITC, was added to each smear and left for 20 min in a moist chamber. After incubation with the conjugate, the smears were rinsed in a jet of PBS and washed for 1 h in a fresh bath of PBS. Finally, the smears were removed from the bath, mopped free of the excess buffer, and one drop of 1:1 glycerol PBS pH 7·6 (this pH being chosen because it enhances optimum FITC fluorescent emission; Fothergill, 1969) was added to each smear and then topped with a No. 1 grade coverslip.

(iv) *Microscopy.* A Leitz Orthoplan microscope was used with transmitted and also incident (Ploem, 1967) light systems with specific waveband interference exciter filters (Rygaard & Olsen, 1969), and 45° inclined (dichroic) mirrors.

(v) *Criteria for positive staining.* Strict criteria were applied. Positive results were recorded only when 25% or more of the total number of cells on the smear showed fluorescent staining at a titre of 1:4 or greater—the end-point of the titration was taken to be the last dilution of the patient's serum that showed these stringent requirements. The baseline titre of 1:4 was found to be necessary to exclude the possibility of non-specific reactions, which were found even in the control serum when it was applied to the cell smears either neat or in a dilution of 1:2. It is emphasized that the results of others now engaged in similar studies elsewhere (de Moragas, 1972, personal communication), who do not dilute the test sera, therefore are not comparable to our own. All positive sera were retested and titrated.

FINDINGS

The patients with halo naevus

Twenty-three patients were studied: fifteen were male: twelve were under the age of 20 years. In four patients the halo naevi were solitary and in two there were only two naevi at the time they were examined and the blood was taken. Eleven patients had vitiligo affecting the skin at sites distant from the naevi but in two of them the patch was solitary and small. Four of these patients had inactive fully resolved naevi, one of which was biopsied to confirm this because it continued to show moderate pigmentation and, although barely palpable, was obviously elevated.

Because of the persistence of depigmented haloes in some patients, it was not always possible to determine clinically whether the main stage of regression of the centrally placed naevus, in cases where these were multiple, had happened in all moles simultaneously or in a piecemeal fashion. Two colleagues reported that the moles on their own skin resolved in about a year, and that the haloes had mostly repigmented by that time.

Immunological tests

(1) *Positive*

Humoral antibody directed against the cytoplasm of malignant melanoma was traced in *every* patient tested with the following diseases:

(i) *Malignant melanoma*—fifty-four, primary, early or localized stages of the disease without metastases. These were from a total of 180 malignant melanoma patients (Phillips & Lewis, 1970).

(ii) *Halo naevus*—fifteen (solitary lesion, four; multiple lesions, eleven).

(2) *Negative*

Humoral antibody to cytoplasm of malignant melanoma cells was not found in any of the patients with the following diseases:

(i) *Halo naevus.*

(a) *After surgical excision of solitary halo naevus*—two. Two boys aged 6 and 9 years, had cytoplasmic antibody titres of 1 in 4 and 1 in 8 respectively. 3 and 5 months after surgical excision of each halo naevus, a further specimen of serum was taken from them both and in neither was antibody found.

(b) *Resolved multiple lesions*—four. Serum from four patients was examined. Two of the sera were from dermatologists who had each once observed many halo naevi on their own skin. In one, the fifteen moles had been subject to an abrupt and marked halo formation, the process having taken about 6 months. The skin had virtually completely repigmented and the central moles were now undetectable. His serum had been taken and examined 6 months after this spontaneous resolution.

(c) *Patients unexpectedly negative*—four. Four patients, two of them with solitary lesions, did not have cytoplasmic antibodies in their sera at sufficiently high titre to be called positive by our strict criteria. Unfortunately none was available for re-assessment, their sera having been sent to us by dermatologists from elsewhere. Two of the sera had been stored for months at +4°C and then at −20°C. The serum of the third had been delayed for a week on its journey from Scotland and was opaque from heavy contamination with micro-organisms. But this serum came from a patient with a solitary halo naevus of several years' duration that had not altered its appearance and in retrospect the referring dermatologist considered that it must have resolved completely years previously. It was interesting to note, therefore, that on several testings of aliquots of this serum, we were tempted to call the result a 'weak positive' at 1 in 4 dilution and at 1 in 2 it was always 'positive'. This proved the value of having on each microscope slide a known positive and a known negative test serum for comparison. The fourth patient had had multiple halo naevi and one patch of vitiligo without a central mole. Although we did not examine this patient ourselves, it seems that the lesions were still actively regressing. This patient, therefore, is the only one in whom we are unable to explain our negative findings.

It is important therefore to judge the activity of the process of resolution of the melanocytic naevus not solely by the state of the depigmented halo. Examination of the mole itself by biopsy may be needed for true assessment of the stage of the inflammatory process. The lymphocytic infiltrate signifies that the mole is regressing actively. Furthermore the central mole may remain slightly elevated and retain some pigment long after the naevus cells have disappeared, mimicking continued activity.

(ii) *Multiple melanocytic naevi*—seven. This group includes two children with acutely erupting moles and one man, a psoriatic, with scores of moles that had become acutely inflamed while an in-patient having intensive dithranol treatment.

Another man aged 20 years, a patient of Dr R.H.Champion, had the strange phenomenon of a nummular eczema overlying nearly all the pigmented naevi but without depigmented haloes developing. This patient corresponds to that of Meyerson (1971).

(iii) *Vitiligo (unassociated with halo naevi)*—forty-seven. One patient (Macmillan & Rook, 1971), a man of 37 with atopic eczema, had the peculiar phenomenon of an elevated red scaly rim to each lesion of the symmetrical widespread vitiligo.

(iv) *Benign juvenile melanoma*—six. These patients each had a biopsy excision of their solitary lesion. Sections of the three lesions in which the diagnosis was doubtful were seen by other dermato-histopathologists (Dr I.Whimster, Dr G.C.Wells, Dr K.V.Sanderson and Dr E.Wilson Jones).

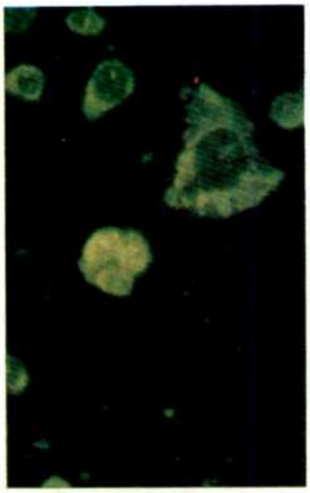
(v) *Suspected malignant melanoma series*—forty-nine. Cytoplasmic staining was negative in every one of the following patients who had been referred with the diagnosis of malignant melanoma but whose lesions were diagnosed later on histopathological grounds to be:

Seborrhoeic warts (multiple and deeply pigmented)	4
Actinic keratoses	2
Pigmented squamous cell carcinoma	3
Pigmented basal cell carcinoma	15
Epidermoid and sebaceous cysts with overlying epidermal hyperpigmentation	6
Histiocytoma with deeply pigmented overlying epidermis	2
Benign blue naevi	2
Multiple mongolian spots	1
Melanocytic naevus (infected or traumatized)	14

RELATIONSHIP OF CYTOPLASMIC ANTIBODY TO RESOLUTION OF THE MOLE AND TO HALO FORMATION

Circulating malignant melanoma cytoplasmic antibody was noted to be closely related to the halo formation in that (i) it was detected in the serum, even when the naevus showing the phenomenon was solitary and small, always provided the central mole was still regressing. When the inflammatory stage was complete and the lymphocytic infiltrate had disappeared, cytoplasmic antibody likewise became undetectable. The mole, however, might still be slightly elevated and pigmented and could appear to be only incompletely resolved: then biopsy might be needed to confirm that the inflammatory process was no longer active. Sometimes, also, the depigmented halo persisted long beyond the time cytoplasmic antibody was detectable in the serum. Thus, after its initial formation, the fate of the halo seemed to be disconnected from the presence of cytoplasmic antibody. (ii) It disappeared from the serum within months of excision in the two cases of solitary halo naevus as would have been predicted, the antibody being an IgG.

PLATES 1–6. Malignant melanoma cell substrate preparations. *Cytoplasmic humoral antibodies* positive indirect test (patient's serum; FITC-labelled goat antihuman IgG; preparation of malignant melanoma cells). (1) High power smear. (2) Low power smear. (3) Single dendritic cell with double nucleus; high power smear. (4) Single dendritic cell; high power 5 day tissue culture. (5) *Plasma membrane humoral antibodies* (patient's serum; FITC-labelled goat antihuman IgG; using the same patient's *own* malignant melanoma cells on a smear). (6) Malignant melanoma cells in (3 day) tissue culture; low power, Fontana-Masson. (Fluorescence microscopy: incident light (epi-illumination) and narrow-band barrier filters.)



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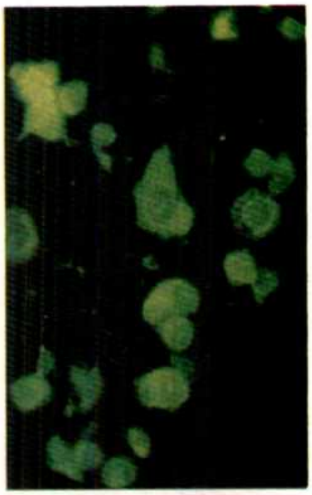

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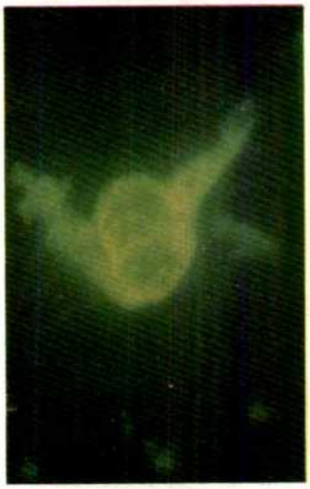

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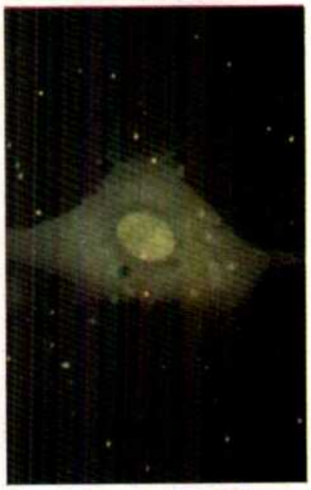

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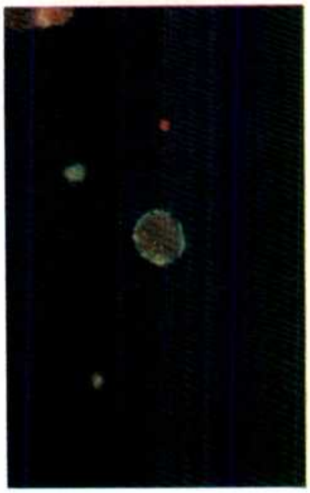

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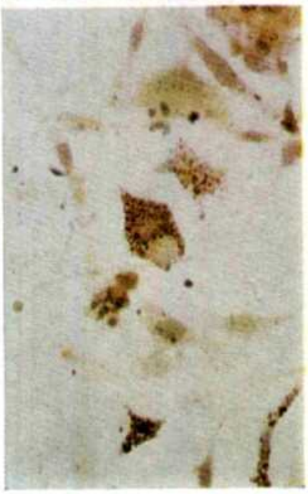

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DISCUSSION

The common cytoplasmic antigen in malignant melanoma has already been established by specific immune blocking and adsorption studies (Lewis *et al.*, 1969; Phillips & Lewis, 1970) to be tumour-specific in early or localized malignant melanoma. That it is not found in sera of patients with different types of naevi or other pigmented skin lesions suggests the possibility that it might be exploited for a diagnostic test. This is being developed by several research groups under the auspices of the melanoma group of the European Organization for Research into the Treatment of Cancer and will be reported later. The main purpose of this communication is to emphasize the findings of positive cytoplasmic antibody titres in the sera of patients with halo naevus.

It is well recognized that malignant melanoma may arise from pigmented naevi and that the number of pigmented moles (Sanderson, 1968) found on the skin alters during the individual's lifetime. Commonly these naevi appear during childhood and resolve in early middle age (Lunde & Stobbe, 1949). Few people of any race do not have some easily detectable moles somewhere on their skin surface (Lewis & Johnson, 1968). Well established malignant melanoma may undergo spontaneous regression (Everson & Cole, 1966) and this phenomenon first suggested the possibility that immune mechanisms might exist in malignant melanoma (Smith & Stehlin, 1965). The situation of malignant melanoma presenting as a secondary deposit in regional lymph nodes where the primary tumour had regressed has been reported from several centres (Das Gupta, Bowden & Berg, 1963; Pack & Miller, 1961; Lewis & Kiryabwire, 1968). The development of a halo of depigmentation around such regressing melanomas has also been seen on occasion (Fig. 3). The case of Ellis (1962) quoted by Everson & Cole (1966) is notable.

When a depigmented halo develops around an otherwise unremarkable mole, immunological mechanisms are suspected to have supervened because:

(1) In the skin: masses of lymphocytes infiltrate the naevus cells of the mole.

(2) In the blood: malignant melanoma cytoplasmic humoral antibodies are detectable. Most moles and most malignant melanomas, however, do not develop noticeably depigmented haloes.

Our discovery of an antigenic link between the halo naevus and malignant melanoma raises the possibility that the phenomenon seen in the benign halo naevus may be a manifestation of a seldom seen interim stage of rejection of an early malignant melanoma arising from such a naevus but which to all other clinical and histopathological appearances seems to be benign. The patient of Shapiro & Kopf (1965) is the only probable exception to this where malignant melanoma appeared to develop in a mole undergoing halo formation.

In the natural history of human malignant melanoma, a fall in antibody titre precedes the clinica appearance of metastases and may be due to the production of a form of idiotypic antibody against the original tumour-specific antibody (Lewis *et al.*, 1971; Copeman, Bleehen & Lewis, 1973) While the primary malignant melanoma is solitary, irrespective of its volume, the antibody titre is elevated: and the tumour remains discrete and there are no metastases. This has focused our attention onto the possibility that rather it is the patient with disseminated and metastatic malignant melanoma who is abnormal because his normal system of surveyance and prevention of disseminated malignancy is in some way broken down. The disparity between the frequency of moles and the incidence of melanomas also supports this.

The halo naevus therefore may be just an exaggerated form of natural protective mechanism in which the whole naevus tumour mass rather than perhaps just a few abberrant cells undergo a dramatic, rapid, and massive rejection. The patient with the malignant melanoma then, particularly if the tumour metastasizes, may be the one who presents the oddity; the halo phenomenon being normal. While not denying the chance that cytoplasmic antibody may be an effect functionally unrelated to the pathogenesis of the signs of halo formation, nevertheless, we believe that these signs are witness of an unusually accelerated phase in the otherwise normal natural history of the mole and malignant melanoma; a phase that without this cutaneous pointer would have escaped notice.

This behaviour of moles and melanomas raises the questions not only why (halo) naevi are spontaneously rejected and heal, but why do malignant melanomas develop and persist in patients who in all other respects appear to have normal intact immunological mechanisms.

IMPLICATIONS

If the cytoplasmic antibody is proven to be cytopathic, which our preliminary studies now indicate it to be, then the halo formation around the halo naevus and the vitiligo at sites distant from the halo naevi can be implied to be the result of the destructive nature of this antibody. The depigmented halo area of epidermis contains few if any functioning melanocytes depending upon the stage when it is examined (Caesarini, Bonneau & Calas, 1968; Ebner & Niebauer, 1968; Stegmaier, Becker & Medenica, 1969) (Fig. 3).

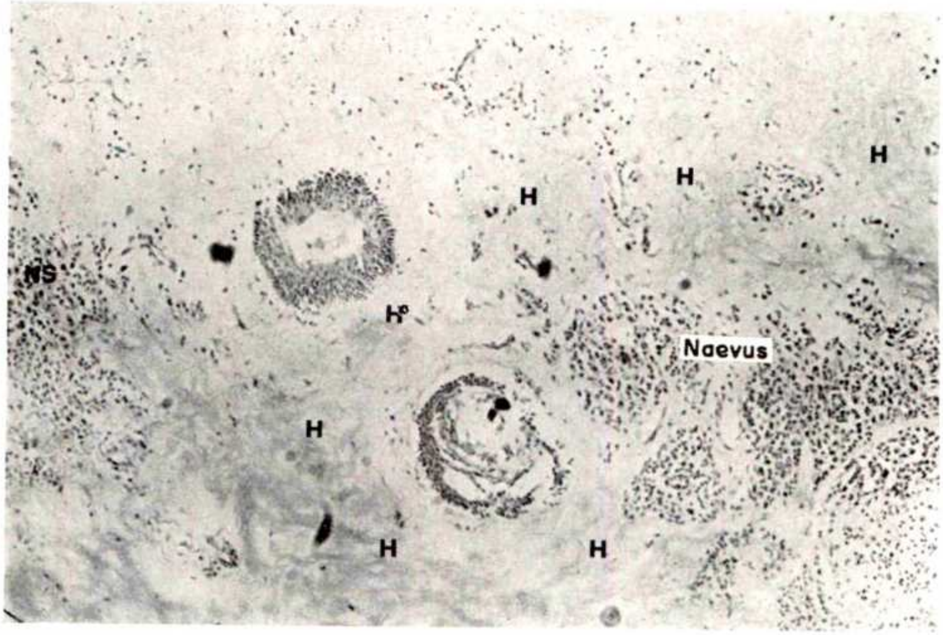

FIGURE 3. Flat-mounted specimen of a segment of halo naevus. NS, normal pigmented skin; H, zone of fully developed depigmented halo: very few melanocytes; Naevus, resolving melanocytic naevus at centre. Low power, Fontana stain.

(1) The process of the disappearance of the melanocytes in the skin of the halo area is concurrent with the disappearance of the naevus cells of the central mole and with the detection of circulating cytoplasmic antibody; it may be that this same antibody destroys both types of cell.

(2) The vitiligo, so commonly seen in halo naevus patients on other areas of the skin away from the moles, is similar clinically and ultrastructurally (J. A. A. Hunter, unpublished data) to the ordinary variety of vitiligo: it may be, then, that there are two pathogenetic types of vitiligo both expressing themselves in the skin similarly: and the aetiology of that type seen with halo naevus could be directly related to the mechanism of the halo formation. Sometimes the vitiligo may precede the halo formation

by years and there may be a positive family history of vitiligo: perhaps, therefore, the patient is merely vitiligo prone (*Lancet*, 1971) as in the common variety, whatever its aetiology (Copeman *et al.*, 1972), and the rising titre of cytopathic cytoplasmic antibody uncovers this latent weakness.

A further important implication of this work, and a by-product of it, is our inability to detect cytoplasmic malignant melanoma antibody in six patients with benign juvenile melanoma. We believe this to be additional evidence (Kopf & Andrade, 1966) that:

(3) Juvenile melanoma is not immunologically related to malignant melanoma.

REPRESENTATIVE CASE HISTORIES

(1) A boy aged 9 years, a patient of Dr P.D.Samman, had had a solitary naevus on his left cheek for perhaps 2 years. Recently it had shown some signs of resolution and had developed a halo. Malignant melanoma cytoplasmic antibody was positive at a titre of 1 in 8. The lesion was surgically excised at the request of his mother because her other child had died of 'a tumour'. The histopathology was completely characteristic for an actively resolving halo naevus (Findlay, 1957; Wayte & Helwig, 1968). 5 months later circulating cytoplasmic antibody was no longer detectable.

(2) A boy aged 6 years had had a single halo naevus on the shoulder for 8 months. The naevus was getting slightly smaller and the halo was enlarging slowly. Malignant melanoma cytoplasmic antibody was positive at a titre of 1 in 4. The lesion was removed at the request of his parents who were going abroad. Histopathological examination showed the characteristic changes of a resolving halo naevus. Another specimen of serum was retested for cytoplasmic antibody 3 months later: it had become negative.

(3) A man aged 28 years, patient of Dr R. Summerly, had eight halo naevi on the chest and back in all stages of involution. While under observation during the last couple of years, he had developed two small patches of vitiligo at sites distant from the naevi. The family history is negative for vitiligo, diabetes mellitus, Addisonian anaemia, and thyroid disease. Organ-specific antibody tests, including thyroid antibodies and parietal cell antibodies, were negative. Malignant melanoma cytoplasmic antibody was positive in the serum at a titre of 1 in 4.

(4) A girl aged 14 years, patient of Dr E.Lipman Cohen, was 'born with seventeen moles' that have been disappearing over the last 1–2 years. Meanwhile the depigmented haloes persist. There is no vitiligo. Malignant melanoma cytoplasmic antibody was detected in the serum at a titre of 1 in 4.

(5) A woman aged 40, patient of Dr Suzanne Alexander, had had fifteen halo naevi that appeared to have been active over a period of about 15 years. These now have all resolved and repigmented. Malignant melanoma cytoplasmic antibody was not detected in the serum.

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It is a pleasure to acknowledge the continuing help and inspiration which Professor Dr R.H.Cormane so freely gives to all of us who use immunohistochemical microscopy in examination of the skin.

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A case of pemphigus vulgaris in association with Addisonian pernicious anaemia

R.M.MACKIE, N.K.SAIKIA AND W.N.MORLEY

University Department of Dermatology, Western Infirmary, Glasgow

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SUMMARY

A case is reported in which the diagnosis of pemphigus vulgaris was followed 4 months later by that of Addisonian pernicious anaemia. The lack of case reports of the co-existence of pemphigus vulgaris and other autoimmune diseases, with the exception of myasthenia gravis, is discussed.

It is now well recognized that patients who develop one disease considered to be autoimmune in origin are at risk as far as development of a second autoimmune process is concerned. This process may be clinically apparent, or may manifest itself subclinically in the form of abnormal immunopathological findings (Burnett, 1962; Anderson, 1963). Since 1964, when Beutner & Jordon reported the presence of antibodies in the sera of patients with pemphigus vulgaris which reacted with material in the intercellular spaces of squamous epithelium, there have been good grounds for considering that pemphigus vulgaris belongs to the autoimmune group of diseases. Despite this, there are as yet few reports in the literature of a second autoimmune process co-existing with pemphigus vulgaris, and we therefore report here the presence of both pemphigus vulgaris and Addisonian pernicious anaemia in a patient in whom the diagnosis of pemphigus vulgaris was established by immunofluorescent studies and who was found 4 months later to have pernicious anaemia.

CASE REPORT

Mr J.R., a 59-year-old plumber, first presented in October 1971 with severe oral ulceration of sudden onset. After initial topical treatment had failed to heal the lesions, a biopsy was taken which showed intra-epidermal clefts, and acantholysis considered to be highly suggestive of pemphigus vulgaris. Indirect immunofluorescent studies were therefore carried out, using the method of Beutner, Jordon & Chorzelski (1968) with human vaginal epithelium as substrate. Antibodies to squamous epithelium were found to be present in the serum at a dilution of 1 in 80, thus establishing the diagnosis of pemphigus vulgaris.

At this point the patient's only clinically apparent skin lesions were those of mild psoriasis from which he had suffered for 40 years, but 3 months after the first appearance of the oral lesions he developed raised urticarial lesions on his trunk and thighs: these did not develop into bullae, but were extremely pruritic and cleared slowly leaving pale brown residual pigmentation. Frank blistering of the skin was not observed at any time, although oral bullae which rapidly progressed to ulceration continued to be troublesome. A biopsy taken from one of the raised erythematous lesions present on the back at this time showed early acantholysis, and also marked eosinophilic spongiosis as described by Emmerson & Wilson Jones (1968). This was therefore considered to be diagnostic of the early stages of pemphigus vulgaris, and indirect immunofluorescence carried out again showed pemphigus antibody to be present in the serum, on this occasion at a dilution of up to 1 in 160.

Laboratory investigations in January 1972 showed a falling serum B_{12} level, the value being at the lower limit of normal on the first test at 140 pg% (normal level 100–1000 pg%) but falling to 78 pg% on the second test 18 days later. Faecal fat and xylose absorption studies gave normal results, making malabsorption an unlikely explanation for this low level, and blood films failed to show a macrocytosis. However, sternal marrow puncture revealed a picture suggestive of early megaloblastic change, diagnex blue and pentagastrin tests showed complete gastric achlorhydria, and tests for gastric parietal cell antibodies were strongly positive. A Schilling test carried out both before and after addition of intrinsic factor confirmed the diagnosis of Addisonian pernicious anaemia. Serum folate levels and protein bound iodine were within normal limits, and no antibodies to thyroid microsomes were present.

To date this patient has not required systemic steroid therapy for control of his oral and cutaneous lesions, as they cleared while he was receiving diphenhydramine hydrochloride 50 mg four times daily, and applying Adcortyl A in orabase to the oral lesions. In addition he is receiving hydroxocobalamin 1000 μg intramuscularly every 2 weeks, and has a normal blood picture and serum B_{12} level. Repeat immunofluorescence carried out while both oral and cutaneous lesions were clinically in remission showed pemphigus antibodies to be present in the serum at a dilution of up to 1 in 40.

DISCUSSION

The co-existence of more than one autoimmune disorder is a well recognized phenomenon, which occurs more frequently than can be attributed to chance alone (Whipple, 1965). This is particularly true in the case of pernicious anaemia which has been shown to be frequently associated with spontaneous myxoedema (Tudhope & Wilson, 1962; Markson & Moore, 1962; Doniach, Roitt & Taylor, 1963). The frequency of this association was emphasized by Tudhope & Wilson (1960) who reported that 10% of a series of patients with spontaneous myxoedema developed frank pernicious anaemia, and a further proportion had low serum B_{12} levels. In addition significant associations have been reported between Addison's disease and chronic thyroiditis (Anderson, 1963).

It is therefore of note that a search of the literature has not revealed reports of pemphigus with autoimmune processes other than myasthenia gravis. Considering the rarity of both these conditions, their co-existence is relatively frequent, as Beutner *et al.* (1968) has reported six examples, and Peck *et al.* (1968), Jablonska (1970) and Ridley (1970) have all reported single cases of this association.

Whittinghame & Mackay (1971) have reported the discovery of the pemphigus antibody, without clinical evidence of the disease, in one patient with systemic lupus erythematosus and two with myasthenia gravis. In their series, eighty-nine other patients with autoimmune disorders were screened for the presence of pemphigus antibody with negative results. Cases of pernicious anaemia were not included in this group.

In this case the detection of pemphigus antibody at a time when no cutaneous lesions other than those of psoriasis were present is worthy of note as this would lend weight to the theory that they play a causative role, and are not the result of epidermal damage as in the case of antibody detectable to cardiac muscle after myocardial infarction (Dressler, 1959).

The fact that this patient has not yet required systemic steroid therapy to control oral or cutaneous lesions suggests that at present this is a relatively mild case of pemphigus vulgaris. It will be interesting to see if a deterioration in the oral and cutaneous lesions is heralded by a rise in antibody titre, and the patient is being monitored for such changes.

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Ultrastructural aspects of pemphigoid

F. GUERRA RODRIGO

Laboratory of Cell Biology, Gulbenkian Institute of Science, Oeiras, and Department of Dermatology, Faculty of Medicine, Lisbon, Portugal

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SUMMARY

Biopsies of skin lesions in different stages of evolution were taken from two patients with pemphigoid and studied by electron microscopy.

Early cytoplasmic changes in the basal keratinocytes and supralaminar cleavage were seen. In the germinative cells of the regenerating skin of the blister floor in the older lesions, an inversion of the polarity of the Golgi apparatus was observed.

The findings are discussed and compared with those observed in dermatitis herpetiformis. It is suggested that the pathomechanism of the early dermo-epidermal cleavage may be similar in both diseases.

Since Lever in 1953 first described 'bullous pemphigoid' as a clinico-pathological entity, several electron microscopy studies have been reported (Charles, 1960; Caulfield & Wilgram, 1965; Wilgram, 1967; Kobayasi, 1967; Braun-Falco & Rupec, 1967; Piérard & Kint, 1968; Jakubowicz, Dabrowski & Maciejewski, 1970; Piérard, Kint & Geerts, 1971). Most of them have been focused on the ultrastructural aspects of the skin blisters. There is little information on the early skin changes before dermo-epidermal cleavage occurs.

The present work is an attempt to reach a better understanding of the pathomechanism of dermo-epidermal separation in pemphigoid. For this purpose, the dermo-epidermal junctional zone (basal cells, basal lamina and papillary dermis) was studied in clinically normal skin and in blisters at different stages, from two patients with pemphigoid. The findings were compared with the observations on regenerating skin, which is present in the floor of the older lesions.

MATERIAL AND METHODS

Two patients with clinically recognizable pemphigoid were studied. The diagnosis was confirmed by histopathological examination and by indirect immunofluorescence tests.

Under the local procaine anaesthesia skin blisters at different stages were taken (Table 1).

Lesions more than 24 h old were protected with watch glasses before biopsy. The material was fixed in 3% glutaraldehyde followed by 2% osmium tetroxide (Millonig's buffer, pH 7·3). After embedding in epoxy resin (Epon 812) ultrathin sections were cut in an ultratome with a diamond knife and double stained with uranyl-acetate and lead citrate. They were observed in an Elmiskop 1 A at 80 kW. One micron survey sections were stained with aqueous toluidine blue.

RESULTS

Particular attention was given to the dermo-epidermal junction zone (basal cells, basal lamina and papillary dermis) of the skin surrounding the blisters. It was compared with regenerating skin in the floor of the oldest lesions. The ultrastructural aspects were similar in the two patients.

In the dermis of the clinically normal skin a mild degree of oedema was observed and some inflammatory cells were occasionally present. The basal lamina was normal. On the contrary, some changes were observed in the basal cells (Fig. 1). There were numerous α-type glycogen particles in the cytoplasm (Fig. 2). Accumulations of mitochondria with a dense matrix, sometimes with bizarre shapes, were often seen. Conspicuous, normal looking Golgi apparatus was seen in supranuclear position.

The surface of the cells was highly convoluted, with pseudopode-like processes. Desmosomes were reduced in number but morphologically normal. In some areas there was a striking absence of half-desmosomes.

TABLE I

Patients		Duration of the lesions (days) and number of biopsies					
Sex	Age (years)	1	2	3	4	5	12
F	75	1	1	1	–	–	–
F	84	2	–	–	1	1	1

In the blisters, dermo-epidermal separation took place between the basal cell membrane and the basal lamina. Sometimes several duplications of the lamina were seen in the floor of the bulla (Fig. 3). In recent blisters, the dermo-epidermal cavity was usually clear, without cellular fragments and few inflammatory cells. Fibrin was not observed. The roof was formed by the whole epithelium. Basal keratinocytes in recent blisters were reasonably preserved (Fig. 4). Cellular degenerative changes progressed quickly, the earliest being mitochondrial lysis and cytoplasmic vacuolization.

In the regenerating skin observed in the floor of the oldest lesions, the basal cells had a large nucleus with prominent nucleolus. In the cytoplasm, well developed cisternae of the granular reticulum and numerous free ribosomes could be seen. However, the most characteristic feature of these cells was the unusual size of the Golgi apparatus and the inversion of its polarity (Fig. 5). This organelle was seen near the basal pole of the nucleus, exceptionally in the equatorial area, in contrast with what is usually observed in normal skin. The basal lamina was often duplicated. In the papillary dermis, fibroblasts, inflammatory cells and clumps of microfibrillar structures separated by oedema were observed.

DISCUSSION

The nosographic individuality of pemphigoid (Lever) is now established on clinical, histological and immunological grounds. Clinical and histological characteristics have been extensively reviewed and are well known. The specificity of the circulating antibodies against the basal lamina zone provides valuable support for the diagnosis and for the hypothesis of an autoimmune aetiology of the disease. However, the immunopathological mechanism of the skin lesions is not yet understood.

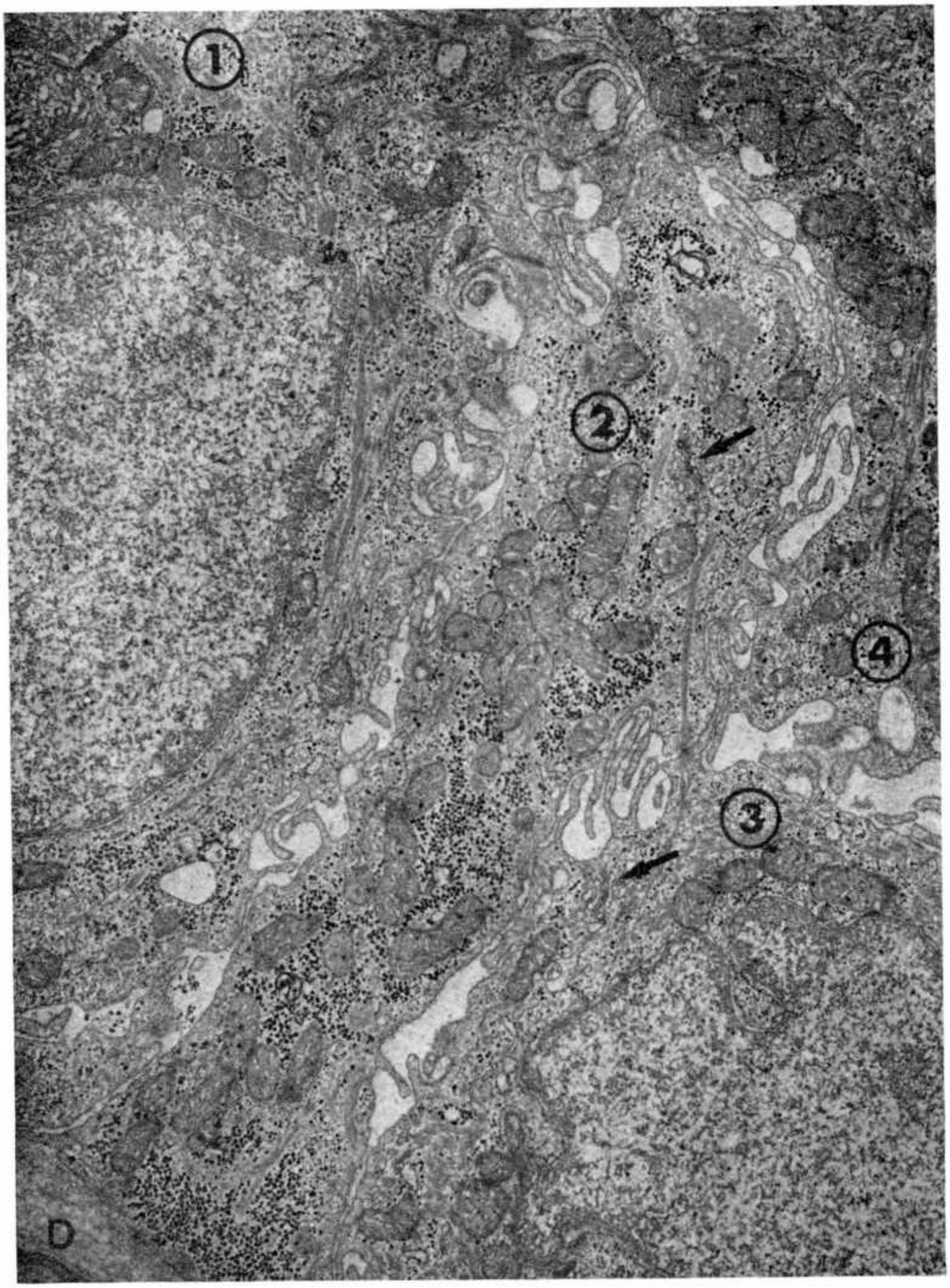

FIGURE 1. Skin surrounding a blister. Four cells can be partially observed. In cells 1–3 the Golgi apparatus can be seen in an apical position (arrows).
Note: glycogen; accumulation of mitochondria (in cell 2); villous surface of the cells with few desmosomes. D, dermis (× 19200).

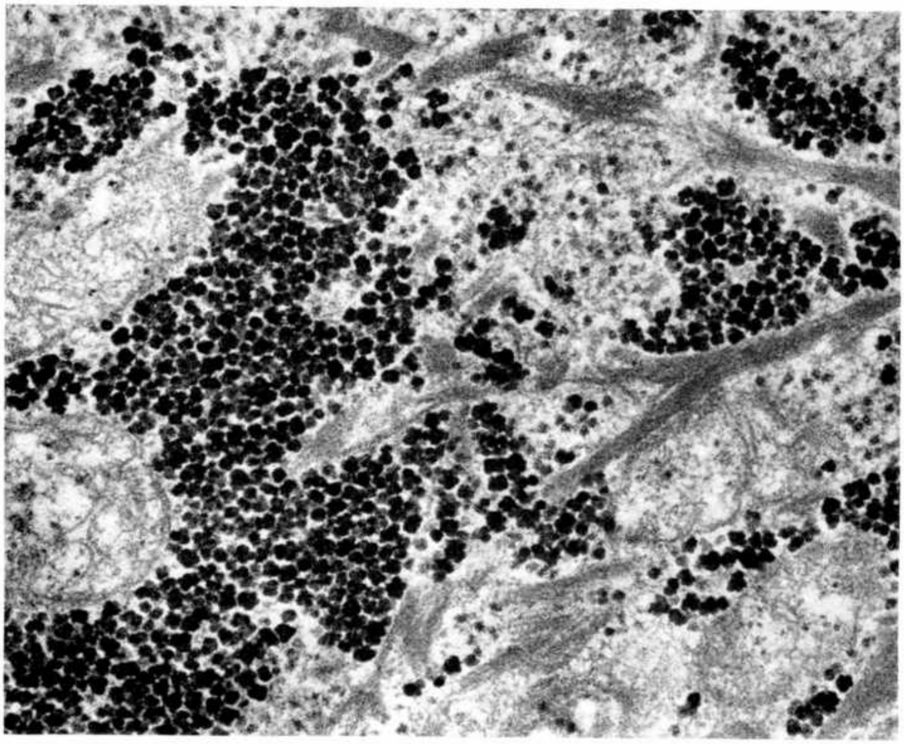

FIG. 2

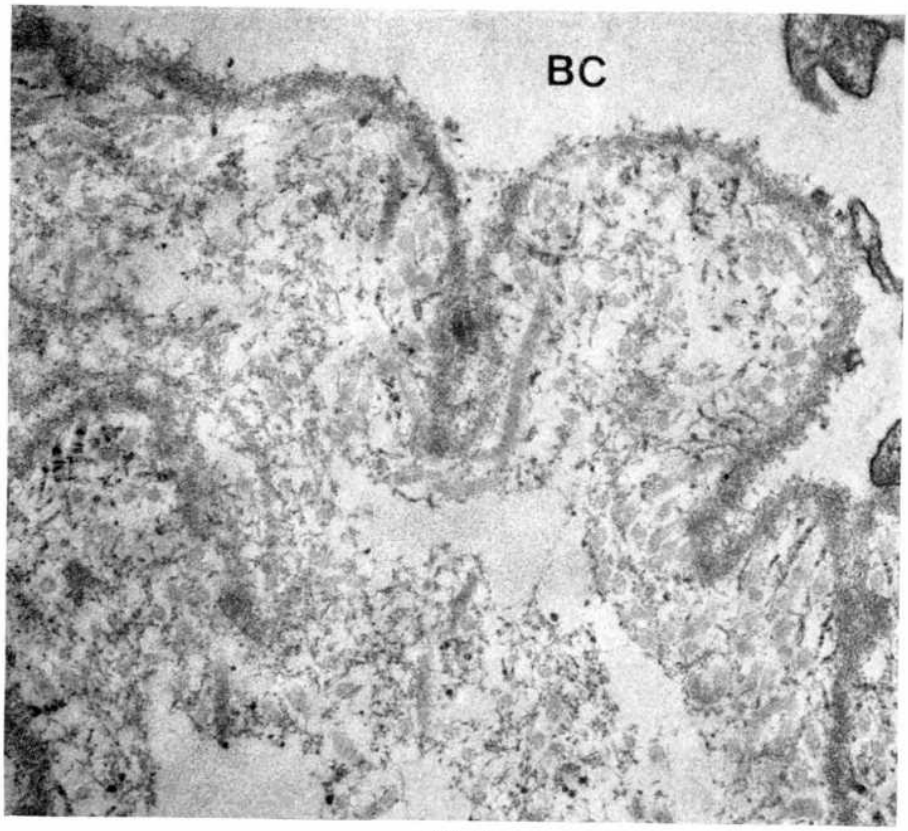

FIG. 3

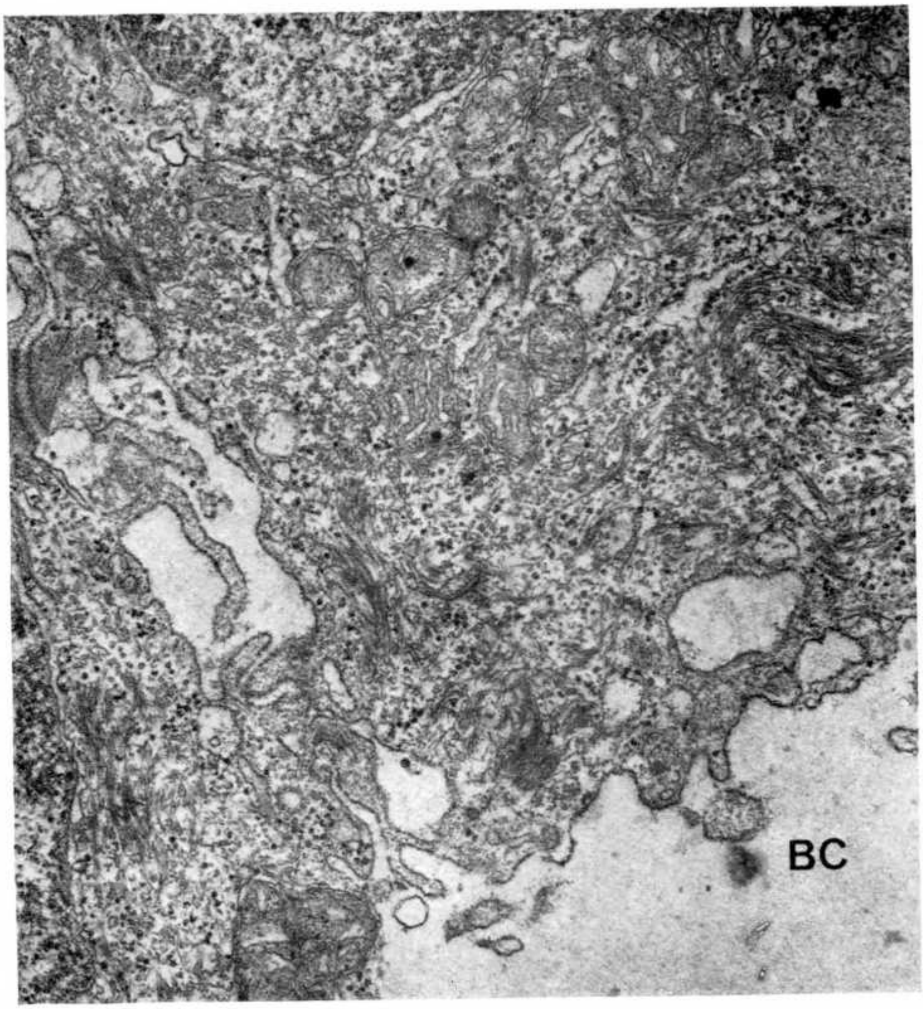

FIGURE 4. Blister roof (less than 24 h). Basal cells are reasonably preserved. Note: distorted mitochondria; no signs of half-desmosomes. BC, blister cavity (× 32000).

The results of ultrastructural studies in pemphigoid and dermatitis herpetiformis have been a controversial subject. In pemphigoid there is now general agreement that the dermo-epidermal cleavage arises between the cell membrane of the basal keratinocytes and the basal lamina. The present findings agree with this interpretation. The observation of Piérard & Kint (1968) of an infralaminar separation in pemphigoid has not been confirmed by recent work by the same authors (Piérard *et al.*, 1971).

In the present work, a change in the cytoplasm of the basal cells in the skin around the blisters was frequently observed. Abundant inclusions of α-particles of glycogen, which are not normally present in epithelial cells, and accumulations of mitochondria, could be seen. In some areas, there was rarefaction

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FIGURE 2. Skin surrounding a blister. Note the aggregates of the glycogen particles (× 54400).

FIGURE 3. Blister floor: duplications of the basal lamina can be seen. BC, blister cavity (× 38400).

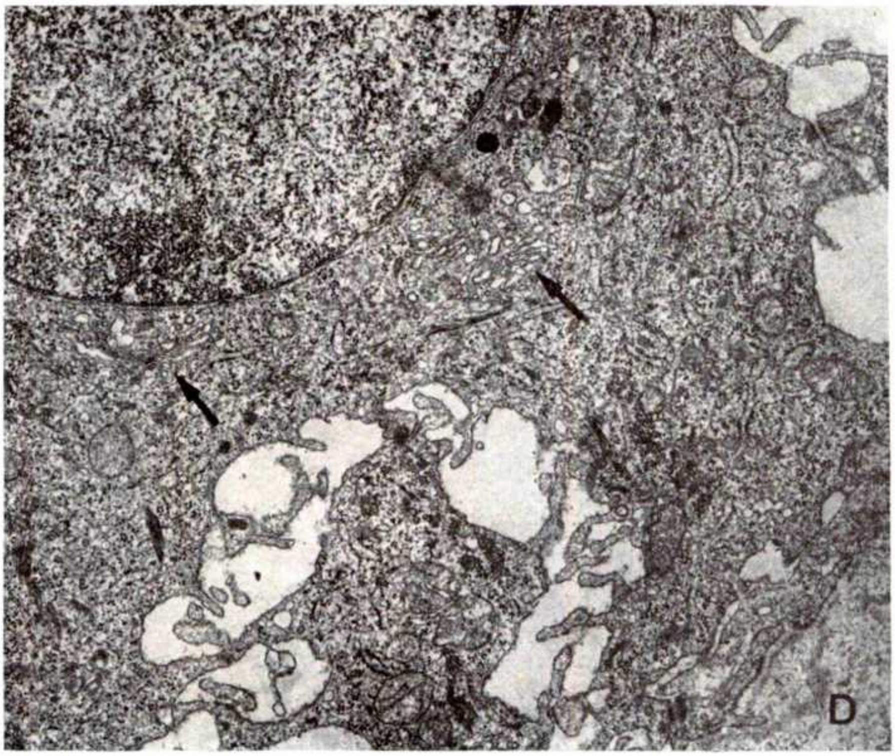

FIGURE 5. Regenerative skin. Note two Golgi apparatus (arrows) in a basal position. D, dermis (× 12800).

of the half desmosomes. These findings suggest that the dermo-epidermal separation may be a secondary phenomenon, presumably caused by the basal cell changes.

The morphology of the basal keratinocytes of the regenerating epidermis was characteristic and quite different. The big nucleus with prominent nucleolus, the abundance of granular reticulum and ribosomes are probably related to the rapid growth and the synthesizing activities of these cells. The most striking finding was however the size and inversion of polarity of the Golgi apparatus. Trelstad (1970) described a similar phenomenon in the chick corneal epithelium during development. This author suggests that the Golgi apparatus is directly involved in the excretion of connective tissue material into the corneal stroma. In the regenerating basal keratinocytes covering the floor of pemphigoid blisters, the observed Golgi inversion may similarly be related to the production and excretion of the structural components of the basal lamina. Recent work has confirmed that this structure is synthesized by the epidermis (Briggaman, Dalldorf & Wheller, 1971).

In a previous work on the ultrastructural aspects of dermatitis herpetiformis, early changes of the basal keratinocytes, and supralaminar separation were observed in pre-blistering skin lesions (Rodrigo, 1972). An immunological phenomenon may be the primary event in both diseases, leading to the same type of supralaminar cleavage. The typical eosinophil and neutrophil infiltrate in dermatitis herpetiformis can be explained by the chemotactic activity of the immunological complexes, which are presumably different (Van der Meer, 1969) from those involved in pemphigoid.

The blister floor in pemphigoid lesions is clearly formed by the basal lamina. Little ultrastructural disorganization is present, even in the late phases of the pathological process, in contrast to what is observed in dermatitis herpetiformis. This fact may be explained by the sparsity of the inflammatory infiltrate in pemphigoid.

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Pharmacology and Treatment

The effect of intracutaneous glucocorticoids on plasma cortisol levels

S.T.ZAYNOUN AND IBRAHIM S.SALTI

Department of Internal Medicine, Divisions of Dermatology and Endocrinology, American University of Beirut and American University Hospital, Beirut, Lebanon

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SUMMARY

The effects of intracutaneous injections of preparations of equal parts of betamethasone disodium phosphate and betamethasone acetate (Celestone Chronodose®) and triamcinolone acetonide (Kenacort-A®) on plasma cortisol levels were studied. Significant suppression of plasma cortisol lasting from 3 to 13 days was observed with various doses of the first preparation. With triamcinolone acetonide, there was only a mild and transient suppression.

The use of intralesional glucocorticoid injections has become an important part of dermatological therapy. The aim is to attain a high concentration of the glucocorticoid at the diseased site in the skin. This type of treatment is used mainly for localized chronic lesions which do not respond to topical applications, even under occlusion, and/or whenever prolonged systemic glucocorticoid therapy is undesirable (Savit, 1957; Guin, Chernosky & Shapiro, 1960; Goldman, 1962; Rebello, 1962). Although intralesional injections would be expected to have smaller systemic side effects, a number of reactions and sequelae have already been reported. These are primarily local reactions such as pain, discomfort, haemorrhage, secondary infection, pigmentary changes and atrophy; and hypersensitivity reactions, varying in intensity from syncope to fatal collapse (Guin *et al.*, 1960; Goldman, 1962). During the past decade, attention has focused on the absorption of glucocorticoids from the site of application or injection and its effects on the pituitary–adrenal axis. It has been shown that topically applied glucocorticoids over extensive areas, under occlusion, lead to significant systemic absorption resulting in adrenocortical suppression (Scoggins, 1962; Kirketerp, 1964; Taylor, Malkinson & Gak, 1965; James, Munro & Feiwel, 1967; Keczkes *et al.*, 1967). Few investigations have been concerned with the systemic effects of intradermally administered glucocorticoids. McGugan, Shuster & Bottoms (1963) have shown that a single intradermal injection of 75–100 mg of triamcinolone acetonide resulted in a significant decrease in plasma 17-hydroxy-corticosteroid levels, while doses of 25 mg resulted in prompt fall in plasma cortisol lasting for 4 days. Recently Porter (1971) studied the effects of intralesional triamcinolone acetonide in cystic acne and found that patients who received 20–50 mg of triamcinolone acetonide intralesionally had suppression of plasma 11-hydroxy-cortico-steroids lasting from 2 to 6 days, while those receiving only 15 mg showed no such suppression.

This study was undertaken to investigate the effects on plasma cortisol of intradermal and subcutaneous injections of Celestone Chronodose®* (from here on referred to as Celestone) and Kenacort-A®† (from here on referred to as Kenacort) in normal subjects.

MATERIALS AND METHODS

Cortisol levels in heparinized plasma were determined in duplicates according to the protein-binding method of Murphy (1967). Forty-three healthy male medical students aged 20 to 25 years were selected. None had any obvious endocrine or skin disease or had previously received any glucocorticoid therapy. The subjects were divided into the following groups according to the mode and dose of treatment.

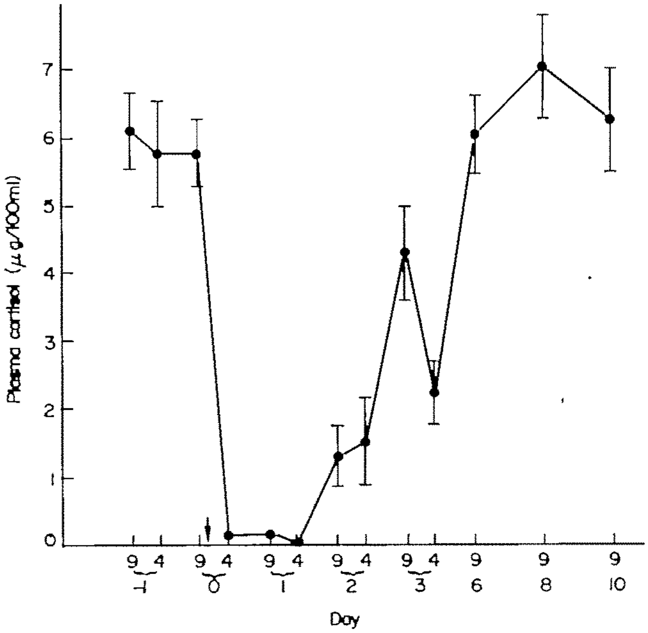

FIGURE 1. Groups I and II: The effect of 1 ml Celestone intradermal injections on plasma cortisol. The mean result ± SEM, for each sampling time is shown. The arrow indicates the injection time.

Group I

Seven subjects received one dose of Celestone, 1 ml intradermally in the arm at 10 a.m. Heparinized blood samples for plasma cortisol determinations were obtained at 9 a.m., on the day preceding the injection, on the day of the injection and on the first, second, third and sixth days following the injection (Fig. 1).

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* Celestone Chronodose, supplied by Schering Corporation, U.S.A., containing aqueous betamethasone disodium phosphate 3·0 mg/ml and betamethasone acetate 3·0 mg/ml.

† Kenacort-A, supplied by Squibb, containing aqueous triamcinolone acetonide 10 mg/ml, 0·75 mg betamethasone acetate is equivalent to 4 mg triamcinolone acetonide.

Group II
Five subjects were treated in a manner identical to those in Group I, but in addition blood for cortisol determination was taken at 9 a.m. on the eighth and tenth days after the injection (Fig. 1).

Group III
Nine subjects received 5 ml of Celestone injected intradermally in multiple sites on the back. Blood samples were taken at 9 a.m. on the day preceding the injection, on the day of the injection and on the first, second, third, sixth, seventh, eighth, ninth and tenth days after the injection (Fig. 2).

Group IV
Four subjects were treated in the same manner as Group III, but blood samples were taken on the third to seventh and on the thirteenth and fourteenth days after the injection (Fig. 2).

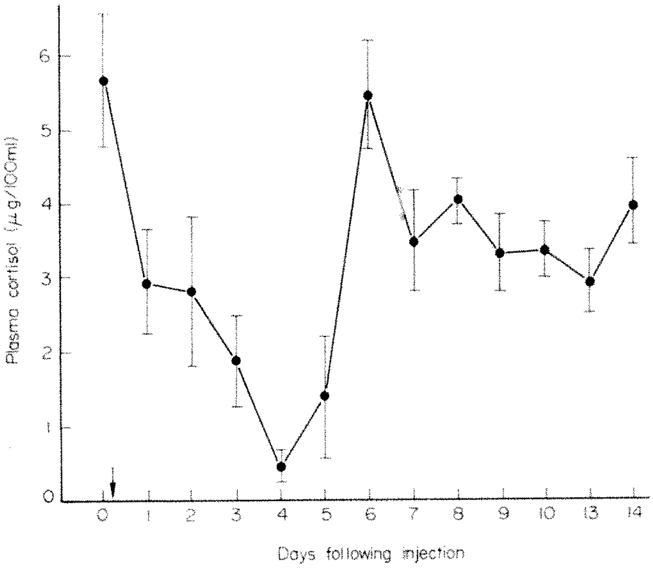

FIGURE 2. Groups III and IV: The effect of 5 ml Celestone intradermal injections on plasma cortisol. The mean result ± SEM for each sample is shown. The arrow indicates the injection time.

Group V
Ten subjects received Kenacort, 5 ml injected intradermally in the back. Blood samples were collected as in Group III (Fig. 3).

Group VI
Two subjects received Celestone, 1 ml subcutaneously in the back. Blood samples were obtained daily at 9 a.m. 1 day before the injection, on the day of the injection and on the first, second, third and sixth to tenth days after the injection (Table 1).

Group VII
Two subjects were treated as in Group VI but received 5 ml Celestone subcutaneously (Table 1).

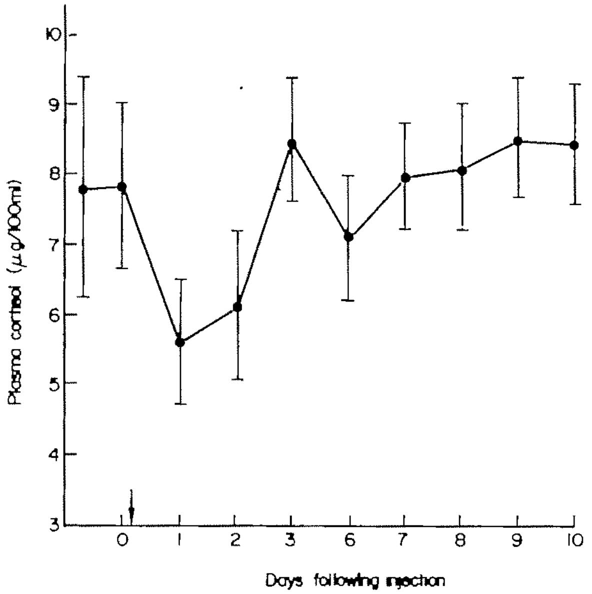

FIGURE 3. Group V: The effect of 5 ml Kenacort intradermal injections on plasma cortisol. The mean result ± SEM for each sample is shown. The arrow indicates the injection time.

Group VIII
Two subjects received Kenacort, 1 ml subcutaneously. Blood for cortisol was taken as described in Group VI (Table 1).

Group IX
Two subjects were injected with Kenacort, 5 ml subcutaneously. Blood samples were taken as in Group VI (Table 1).

TABLE 1. The effect of subcutaneous injections of Celestone + Kenacort on plasma cortisol (μg/100 ml)—Groups VI, VII, VIII and IX

	Days									
	−1	0*	1	2	3	6	7	8	9	10
Celestone,	2·8	3·2	0·0	0·0	0·0	4·4	3·1	1·2	3·8	4·7
1 ml (Group VI)	7·0	2·2	0·0	0·0	3·2	0·2	0·5	0·4	2·4	1·9
Celestone,	3·9	0·4	0·0	0·0	0·0	0·0	0·0	0·0	0·7	1·9
5 ml (Group VII)	4·4	4·3	0·0	0·0	0·0	0·0	0·0	0·0	0·3	1·2
Kenacort-A,	4·7	3·0	5·0	5·6	5·4	3·7	4·0	3·6	5·7	3·4
1 ml (Group VIII)	5·8	4·8	4·0	6·8	5·3	5·0	4·1	3·6	—	5·9
Kenacort-A,	7·9	4·8	0·0	0·0	2·4	3·0	0·1	3·6	3·5	4·8
5 ml (Group IX)	4·2	6·1	0·0	2·4	4·7	2·7	1·6	3·4	3·7	4·2

* Injection given at 10 a.m. All samples taken at 9 a.m.

RESULTS

(1) Celestone, 1 ml intradermal (Groups I and II): There was significant suppression of plasma cortisol as early as 6 h and lasting for 3 days after the injection (Fig. 1). Thereafter cortisol levels returned back to pretreatment values.

(2) Celestone, 5 ml intradermal (Groups III and IV): There was marked suppression of plasma cortisol during the first 4 days after the injection (Fig. 2). Although starting with the fifth day and especially on the sixth day there was a rise in cortisol levels back to normal, the levels on the seventh to thirteenth days after the injection were significantly lower than pretreatment levels.

(3) Kenacort, 5 ml intradermal (Group V): There was a mild and barely significant suppression of plasma cortisol during the first 2 days after the injection. Thereafter plasma cortisol levels were the same as pretreatment levels from the third to the tenth days after the injection (Fig. 3).

(4) Subcutaneous Celestone injections (Groups VI and VII): With 1 ml injections there was a fall of plasma cortisol for at least 3 days, while with 5 ml injections the fall was sustained for 9 days (Table 1).

(5) Subcutaneous Kenacort injections (Groups VIII and IX): With 5 ml injections there was a transient suppression of plasma cortisol which was obvious in the first 2 days (Table 1).

DISCUSSION

In this study we were concerned mainly with the effects of intralesional Celestone administration on plasma cortisol. The preparation contains equal parts of a short acting, rapidly absorbed glucocorticoid (betamethasone disodium phosphate) and a long acting, slowly absorbed glucocorticoid (betamethasone acetate). The results indicate that even with a single 1 ml intradermal injection (containing 3 mg of each of the above mentioned steroids) there was suppression of plasma cortisol during the first 3 days after injection. It is likely that this early suppression is the result of the rapid absorption of the short acting component. With larger 5 ml intradermal injections of the same preparation, there was a similar early suppression of plasma cortisol but in addition there was delayed suppression from the seventh to the thirteenth day. It will be noted that the plasma cortisol levels in the group receiving the larger dose (5 ml) had not reached the same very low levels that were observed in the first 3 days in the subjects that received the smaller dose (1 ml). The reason for this finding is not clear. It is unlikely that the absorbed drug is measured as cortisol by the method used in this study as it was previously shown that betamethasone does not interfere with the specificity of this method for the measurement of cortisol (Murphy, 1967). As expected, subcutaneous injections of Celestone have resulted in marked suppression of plasma cortisol and this suppression was more prolonged with the larger (5 ml) dose. However, the small number of subjects used does not permit statistical analysis of the results.

It is evident that intradermal injections of Kenacort which contain only a long acting, slowly released glucocorticoid (triamcinolone acetonide) resulted in only a transient and less marked suppression of plasma cortisol. A similar pattern was also seen with the subcutaneous injections of this preparation.

Although we have not studied the effect of repeated intradermal injections of either preparation, it seems reasonable that, at least with Celestone, these are expected to result in more sustained suppression of plasma cortisol and hence in potentially clinically significant suppression of the hypothalamic–pituitary–adrenal axis. Moreover, the results of this study indicate the degree of absorption of glucocorticoids administered intracutaneously in normal skin. This may not reflect the degree of absorption from diseased skin.

In conclusion, it is recommended that glucocorticoid preparations which are intended mainly for intradermal use, be composed only of slowly released glucocorticoid components. Moreover, even with such preparations the risk of systemic absorption, though minimal, should be taken into consideration especially when repeated intralesional administrations are necessary.

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Oral β carotene therapy in actinic reticuloid and solar urticaria

FAILURE TO DEMONSTRATE A PHOTOPROTECTIVE EFFECT AGAINST LONG WAVE ULTRAVIOLET AND VISIBLE RADIATION

A.KOBZA, C.A.RAMSAY* AND I.A.MAGNUS

Department of Photobiology, Institute of Dermatology, London, E9 6BX

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SUMMARY

It is very difficult to find effective, acceptable protective agents for patients who are sensitive to long wave ultraviolet (UV) and visible radiation. It has recently been reported that oral *β* carotene is a useful photoprotective agent in erythropoietic protoporphyria (EPP) where sensitivity to visible light is important. The action of this drug has therefore been investigated in other diseases where sensitivity to the same part of the spectrum exists. Two patients with actinic reticuloid and three with solar urticaria, all sensitive to long wave UV and visible radiation were investigated before, during and after therapy with *β* carotene. Detailed monochromator tests were carried out and serum carotene and vitamin A levels determined at regular intervals. Therapy was continued from 2 to 12 months.

Results showed that there was no objective or subjective evidence of improvement in any of the patients despite high serum carotene levels. Large variations in the dose of radiation required to produce a reaction were seen in all patients and it is essential to take account of this variation when assessing any photoprotective agent. It is possible that the mechanism of photosensitivity in actinic reticuloid and solar urticaria is quite different from that in EPP thus explaining the difference in the reported results of *β* carotene in the last disease.

Patients sensitive to long wave ultraviolet (UV) and visible radiation present a difficult management problem because of the lack of acceptable and effective screening compounds.

Conditions which produce sensitivity to the longer wavelengths include porphyria (Magnus, 1968), some cases of solar urticaria (Ive, Lloyd & Magnus, 1965) and actinic reticuloid (Ive *et al.*, 1969). Sensitivity in the latter two conditions is not necessarily limited to the longer wavelengths, indeed in actinic reticuloid there is in addition usually marked sensitivity to terrestrial sunburn wavelengths (290–320 nm). Effective topical screening compounds for this part of the spectrum exist but these sunscreens are ineffective against longer wavelengths. Zinc oxide or titanium dioxide are very effective

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Requests for reprints: Dr C. A. Ramsay, Department of Photobiology, Institute of Dermatology, London, E9 6BX.

photoprotective agents for long wave UV and visible parts of the spectrum but they are usually conspicuous when used on the skin. The addition of compounds to change the colour make them more cosmetically acceptable especially to female patients. However, we have found it difficult to persuade most male patients to use these reflectant sunscreens even when it is explained that they are the only protective materials available.

The reports by Mathews-Roth *et al.* (1970a, 1970b) of photoprotection from oral β carotene in erythropoietic protoporphyria (EPP) are of interest. As β carotene seemed to be of benefit in EPP we felt that it might help patients with other photodermatoses secondary to longwave UV and visible radiation. This paper presents our experiences with five patients, two with actinic reticuloid and three with idiopathic solar urticaria.

METHODS

Monochromator tests

Patients were tested with an irradiation monochromator (Magnus *et al.*, 1959). The back of the trunk was used to determine the minimal erythema dose (MED) or the threshold for an abnormal response, i.e. the lowest dose in a graded series which produced a reaction at individual wavelengths between 300 nm and 700 nm. Irradiated sites were examined at frequent intervals for up to 48 h. These tests were performed a number of times before, during and after β carotene therapy. In the patients with solar urticaria, assessment was made by regular testing at 500 nm and also at 380 or 400 nm. The patients with actinic reticuloid were assessed by regular testing at 380 nm. This wavelength was chosen because both patients showed a reproducible reaction when tested although it was appreciated that absorption of β carotene is less at this wavelength than at 400–500 nm.

Arterial occlusion test

To determine whether an intact circulation was necessary for the photoreaction the forearm was irradiated 15 min after the circulation had been occluded with a sphygmomanometer cuff. The dose was twice the minimum for the reaction with the circulation intact.

Serum vitamin A and β carotene estimations

These were carried out by the methods described by Varley (1967).

Tests of porphyrin metabolism

The methods used were those of Rimington (1958, 1961), Rimington & Cripps (1965) and Rimington *et al.* (1963).

Absorption spectrum of β carotene

Two preparations of β carotene supplied in capsules were used, one from U.S.A. and one from Switzerland. The contents of a capsule of each preparation were dissolved in water at a concentration of 100 μg/ml. In addition, pure β carotene (Sigma Chemical Co. Ltd) at a concentration of 5 μg/ml in petroleum spirit (40–60) was similarly prepared. The solutions were examined in a Unicam SP.800 recording spectrophotometer.

CASE HISTORIES

Case 1—G.H. (G.1809) male 60—*actinic reticuloid*

1948. Dermatitis L. politeal fossa for 2 years. Cause undetermined.

1961. Dermatitis in exposed areas. Patch test to Araldite epoxy resin positive, rash cleared when exposure ceased.

1967. Photosensitive eruption noted with erythema and marked lichenification of exposed areas. Prednisone 40 mg daily started and slowly reduced to present dose of 15–20 mg daily.

Monochromator tests showed lowered MED and abnormal morphological responses at all wavelengths between 300 and 500 nm.

Oct. 1970. β carotene started, dose varying between 15 and 60 mg daily, depending on serum levels.

Nov. 1971. β carotene stopped.

Case 2—C.T. (M.54969) male 64—*actinic reticuloid*

1940. Transient dermatitis on hands recurring occasionally over the years.

1967. Photosensitive eruption commenced, erythema, scaling and lichenification of exposed sites. His job was supervision of tar and bitumen road surfacing. Prednisone 40 mg daily started and reduced to 7·5 mg alternate days which was the dose whilst taking β carotene.

Monochromator tests showed lowered MED and abnormal morphological responses at all wavelengths between 300 and 600 nm.

Patch tests to wood and coal tars were negative and photopatch tests in which two treated areas and one control were irradiated with 'black light' showed only an 'irritant' reaction to coal tar. In the latter test the four 20 W, 2 ft tubes were 27 cm from the skin and the exposure time was 10 min.

Jan. 1971 β carotene started, dose varying between 25 and 150 mg daily.

Sept. 1971 β carotene stopped.

Case 3—F.K. (M.99385) male 61—*solar urticaria*

May 1971. 10 min solar exposure produced erythema and irritation. Negative drug history.

Monochromator tests showed wealing 300–600 nm, maximal at 500 nm. Erythema present immediately after irradiation with four 20 w, 2 ft 'warm white' fluorescent tubes 30 cm from the skin after 5 min exposure. Wealing occurred after 20 min.

June 1971. β carotene started, dose varying between 75 and 100 mg daily.

Nov. 1971. β carotene stopped.

Case 4—A.M. (M.84035) male 23—*solar urticaria*

Aug. 1968. A few minutes of solar exposure produced irritation, erythema and sometimes wealing. One severe exposure gave generalized erythema, faintness and tightness in the chest. Drug history—various antihistamines for hay fever.

Monochromator tests showed wealing 380–500 nm, maximal at 400 nm.

Feb. 1971. β carotene started, dose 30–100 mg daily.

Nov. 1971. β carotene stopped.

Case 5—A.S. (P.1691) female 63—*solar urticaria*

1969. 5 min sunlight gave erythema and swelling.

Drug history—Amesec (aminophylline, ephedrine, amylobarbitone) and prednisone 2·5 mg b.d. for bronchial asthma since 1961.

Monochromator tests showed wealing 307·5–500 nm, maximal at 340 nm.

Aug. 1971. β carotene started, dose varying between 15 and 90 mg daily.

Oct. 1971. β carotene stopped.

TABLE 1. Case 1—actinic reticuloid

Date	Serum β carotene (μg/100 ml)	Serum vitamin A (μg/100 ml)	380 nm threshold* (J/cm^2)
6 Oct. 1970	174	—	1·6
9 Oct. 1970	175	—	—
12 Oct. 1970	—	—	1·7
16 Oct. 1970	—	30	1·3
22 Dec. 1970	1215	47	3·6
19 Jan. 1971	1060	32	2·2
16 Feb. 1971	840	49	2·2
14 April 1971	585	24	7·3
6 June 1971	600	65	1·1
2 Aug. 1971	560	36	7·3
25 Nov. 1971	670	80	—
21 Dec. 1971	360	47	—
13 Jan. 1972	340	32	0·8
21 March 1972	200	—	1·6

* 'Papule' or 'erythema' if this was lower.
—, Not done.

RESULTS

These are shown in Tables 1–5. Tests carried out whilst the patients were taking β carotene are shown between the lines, and it will be seen from the monochromator tests that there was no consistent evidence of improvement in any of the patients. There was no correlation between serum β carotene levels and the threshold for photosensitivity. Although in Case 3 for example there was a striking

TABLE 2. Case 2—actinic reticuloid

Date	Serum β carotene (μg/100 ml)	Serum vitamin A (μg/100 ml)	380 nm erythema threshold (J/cm^2)
25 Jan. 1971	—	—	3·6
27 Jan. 1971	80	—	—
28 Jan. 1971	—	—	9·6
29 Jan. 1971	70	20	—
12 March 1971	520	—	—
25 March 1971	—	—	5·2
24 April 1971	940	90	1·6
2 June 1971	355	—	3·1
22 June 1971	352	26	3·6
16 Sept. 1971	620	—	—
30 Sept. 1971	300	—	—
5 Nov. 1971	140	—	—
31 Jan. 1972	85	—	12·5
21 March 1972	—	—	3·1

—, Not done.

TABLE 3. Case 3—solar urticaria

Date	Serum β carotene (μg/100 ml)	Serum vitamin A (μg/100 ml)	Wealing threshold (J/cm^2)	
			400 nm	500 nm
7 May 1971	—	—	2·0	0·4
8 May 1971	—	—	—	4·4
24 May 1971	100	35	—	—
21 June 1971	100	20	0·7	0·5
12 July 1971	540	40	6·8	715
9 Aug. 1971	780	—	1·7	8·7
6 Sept. 1971	600	90	5·7	14·5
4 Oct. 1971	620	100	2·8	2·9
1 Nov. 1971	460	43	0·6	1·1
29 Nov. 1971	580	—	1·0	14·5
9 Feb. 1972	160	—	1·3	0·7

—, Not done.

increase in threshold 3 weeks after starting treatment this was not maintained. Similarly, there is no consistent pattern in the results of the other cases. Furthermore, in Case 2 the highest threshold for photosensitivity was 3 months after therapy had been stopped when the serum β carotene was normal.

Cases 1 and 3 reported some decrease in burning sensation after sun exposure whilst taking β carotene but this improvement was not maintained. No significant change was detected in the eruption in the patients with actinic reticuloid. Marked sensitivity to the 300–320 nm spectrum existed in the patients with actinic reticuloid and no change in this was seen on regular testing during β carotene therapy.

TABLE 4. Case 4—solar urticaria

Date	Serum β carotene (μg/100 ml)	Serum vitamin A (μg/100 ml)	Wealing threshold (J/cm^2)	
			400 nm	500 nm
16 Feb. 1971	90	36	4·1	10·3
17 Feb. 1971	—	—	—	3·4
19 Feb. 1971	—	—	—	3·3
24 Feb. 1971	75	—	—	—
22 April 1971	305	50	75·9	6·0
1 June 1971	570	30	79·1	9·0
24 Aug. 1971	760	42	7·2	9·3
21 Sept. 1971	575	43	3·1	715·5
14 Oct. 1971	560	86	0·9	713·5
24 Nov. 1971	520	106	0·5	1·6
4 Feb. 1972	205	—	1·6	7·8

—, Not done.

TABLE 5. Case 5—solar urticaria

Date	Serum β carotene (μg/100 ml)	Serum vitamin A (μg/100 ml)	Wealing threshold (J/cm^2)	
			380 nm	400 nm
10 Aug. 1971	145	28	0·4	1·9
11 Aug. 1971	—	—	0·4	0·6
8 Sept. 1971	720	36	0·5	1·1
6 Oct. 1971	780	40	0·6	0·9
2 May 1972	150	22	2·5	1·6

—, Not done.

Arterial occlusion test

This was carried out in four patients (Cases 1 and 3–5). In two of the patients with solar urticaria (Cases 3 and 4) wealing appeared a few minutes after arterial occlusion was released thus showing that an intact circulation during irradiation was not essential for the reaction to develop. In the other patient with solar urticaria (Case 5) no wealing occurred when occlusion was released. A similar result was found in Case 1, a patient with actinic reticuloid. Arterial occlusion during irradiation at 380 nm completely prevented the delayed papular response which appeared normally on the unoccluded opposite control arm. Case 2 was unable to tolerate arterial occlusion.

Tests of porphyrin metabolism

All patients had negative screening tests on red cells, urine and faeces. In the patients with solar urticaria (Cases 3–5) quantitative porphyrin tests on red cells and urine were normal.

Absorption spectrum of β carotene

This is shown in Fig. 1.

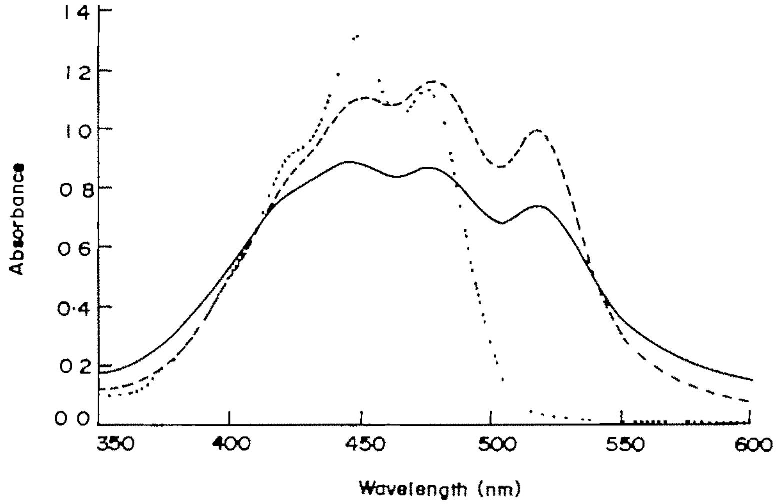

FIGURE 1. Absorption spectra of β carotene. . . ., 5 μg/ml β carotene (pure); - - -, 100 μg/ml U.S.A. preparation; —, 100 μg/ml Swiss preparation.

DISCUSSION

There is no doubt that diseases associated with sensitivity to long wave UV and visible radiation present great problems both for the patient and for the physician, and it is therefore essential to explore any possible mode of therapy. The reports by Mathews-Roth *et al.* (1970a, 1970b) suggested that oral β carotene was effective in EPP where sensitivity is most marked at 400 nm and it therefore seemed reasonable to try this drug in other photodermatoses with sensitivity to this part of the spectrum.

Much research on the photoprotective effects of various carotenoids has been carried out in bacteria. Sistrom, Griffiths & Stanier (1956) put forward the hypothesis that carotenoids, in the bacterium which they studied, act as chemical buffers against photo-oxidation by chlorophyll. Bacteria from which coloured carotenoids has been removed were sensitized by toluidine blue to visible light (Kunisawa & Stanier, 1958). Similar results were obtained in non photosynthetic bacteria by Mathews & Sistrom (1959). The species distribution of carotenes in bacteria would suggest that their prime function is to protect against photodynamic killing (Krinsky, 1968). Further investigations have shown that the number of conjugated double bonds in the carotenoid is critical from the point of view of its photoprotective capacity, it seems that in bacterial carotenoids at least nine conjugated double bonds are essential (Mathews-Roth & Krinsky, 1970a). The photoprotective effect of carotenoids is not limited to micro-organisms. Mathews (1964) showed that injected β carotene protected mice against lethal photosensitization by haematoporphyrin. She suggested that β carotene might be useful in human photosensitivity to visible light. In fact Kesten (1951) had already reported the apparent successful use of this drug in a female patient with solar urticaria, who (at 44) had suffered the disorder since the age of 5. Tests with Corning filters, showed her to be sensitive between 420 and 490 nm, i.e. a similar action spectrum to our Case 4. Carotene cream applied locally reduced the reaction to artificial light and the patient was then given carotene orally. Re-testing produced no reaction and this protection was thought to be due to the oral carotene. However, one very important factor which was not considered by Kesten (1951) is the variation in dose of light which is needed to produce a reaction in solar urticaria (Ive *et al.*, 1965). This point is illustrated very clearly in our results (Tables 1–5). If we had not carried out more than one test before starting β carotene we could easily have misinterpreted the results and arrived at a completely different conclusion. Past experience has repeatedly shown us that in patients with EPP and idiopathic solar urticaria reactivity of the skin to radiation varies considerably in the absence of any treatment. Natural variation, which also occurs in normal sunburn reactions (Rottier, 1962), must be borne in mind when assessing any form of therapy. Furthermore, the intensity and spectral distribution of artificial sources are subject to variations which may be overlooked unless they are repeatedly calibrated.

In 1970 Mathews-Roth *et al.*, reported the beneficial results of treatment with β carotene in three patients with EPP. In addition to marked subjective improvement in symptoms of photosensitivity these patients also showed improvement when tested with filtered artificial sources. These results suggested that oral β carotene might be of benefit in other diseases when sensitivity to long wave UV and visible radiation is important.

We decided that any case chosen for the trial of β carotene therapy must show abnormal monochromator tests which could be repeated on several occasions as it was felt that this was the only way in which an objective assessment of results could be made. Porphyria cutanea tarda (PCT) where sensitivity in the region of 400 nm is present can now be treated very successfully by phlebotomy (Epstein & Redeker, 1968). It was therefore decided that patients with PCT should not be included. During this time a number of patients with EPP, were seen but none showed abnormal monochromator tests and were therefore excluded.

Our investigation was limited to three patients with solar urticaria and two patients with actinic reticuloid. In this latter group sensitivity was present to many wavelengths between 300 and 600 nm (Ive *et al.*, 1969). It was felt that if a suitable oral photoprotective agent could be found for the long UV and visible light sensitivity then the sensitivity in the normal 'sunburn' range could probably be managed with conventional topical therapy.

There was little in the way of side effects attributable to β carotene in these patients. Case 5 described a severe exacerbation of hay fever which lasted considerably longer than her usual attacks and which occurred at a time of the year when she was usually unaffected. Case 2 complained of recurrent swelling of the tongue and face. It is impossible to make an objective assessment as to whether these were true side-effects or not and are mentioned for reference to future trials of this drug.

It has been stated (Goodman & Gillman, 1965) that humans are inefficient at converting β carotene to vitamin A, but with an upper limit of normal for serum vitamin A of 50 μg/100 ml (*Geigy Scientific Tables*, 1970), then it will be seen from Tables 1–5 that four of our patients (Cases 1–4) had raised vitamin A levels at some time during the course of the trial. If the reports of Mathews-Roth *et al.* (1970a, 1970b) are confirmed and β carotene becomes the standard treatment for EPP then it would be advisable for serum vitamin A levels to be checked at regular intervals because of the known toxic effects of hypervitaminosis A (Goodman & Gillman, 1965).

It is obvious from our results that β carotene conferred no definite photoprotection in the five patients studied and we should try to analyse the possible reasons for this negative result.

(1) *Drug not given for long enough*

This seems unlikely. Mathews-Roth *et al.* (1970a, 1970b) reported improvement in their patients with EPP after less than 1 month of therapy. Our patients received the drug for periods varying from 2 to 12 months.

(2) *β carotene levels were not high enough*

Again this seems unlikely. All patients had serum carotene levels in excess of 500 μg/100 ml for most of the trial period. This of course does not necessarily mean that cellular levels were high but all patients were clinically carotenaemic. Serum levels in our patients were generally higher than those reported by Mathews-Roth *et al.* (1970a, 1970b) where successful photoprotection was achieved in EPP.

(3) *Mechanism of photosensitivity is different from that in porphyria*

At first sight this might appear to offer the most likely explanation although the exact mechanisms underlying photosensitivity in solar urticaria, actinic reticuloid and porphyria are unknown. However, it is known that in porphyria for a reaction to develop in the skin an intact circulation (and by inference, perhaps oxygen) is essential during the time of irradiation (Rimington *et al.*, 1967). Similarly, in some cases of solar urticaria the reaction is dependent upon an intact circulation (Brunsting & Epstein, 1957). In other cases it is the reverse (Blum, 1964). Two of our patients with solar urticaria (Cases 3 and 4) developed wealing whether or not the circulation was occluded but in the other patient (Case 5) an intact circulation during irradiation was essential. Of particular interest was Case 1, a patient with actinic reticuloid. Occlusion of the circulation during irradiation completely prevented the abnormal papular reaction which normally appeared a few hours later. As far as we are aware this is the first time that the effect of arterial occlusion on the irradiation reaction has been reported in actinic reticuloid.

It is possible that the photosensitivity in porphyria is an example of photodynamic action (Magnus, 1968) with the formation of singlet or $^1\Delta_g$ oxygen (Dalton, McAuliffe & Slater, 1972). In solar urticaria whilst some cases may have a similar mechanism, in others the photosensitivity appears to be due to other processes apparently not dependent on oxygen (Blum, 1964). There is evidence that β carotene quenches singlet oxygen (Foote & Denny, 1968) and these authors suggested that this might explain the role of β carotene in plants. It could be argued that photoprotection was not achieved in our Cases 3 and 4 with solar urticaria because their reaction was not dependent on oxygen. However, in the other patient with solar urticaria (Case 5) and in one patient with actinic reticuloid (Case 1) the reactions did appear to be oxygen dependent as judged by the arterial occlusion test and β carotene had no protective action in these patients. Possibly compounds other than oxygen carried by the circulation, not influenced by β carotene, are essential for the reaction in Cases 1 and 5. It should also be pointed out that tests in Cases 1 and 2 were carried out at 380 nm, one of the lower points in the absorption spectrum of β carotene (Fig. 1).

Another problem which arises in assessment of any therapy in actinic reticuloid is the variation in severity of the disease which may occur at different times of the year. However, one of our patients, Case 1, was treated with β carotene continuously for 12 months and there was no significant change in the clinical appearance during this time. In addition it will be seen from Table 1 that the highest threshold dose (7·3 J/cm^2) was obtained in April and August of the year of treatment but that in July of the same year the threshold dose was very low (1·1 J/cm^2). Case 2 was treated for 9 months, from January to September without any clinical improvement, and the highest threshold dose was obtained in March (5·2 J/cm^2) although this was lower than one pretreatment threshold dose (9·6 J/cm^2). These results stress again that variations in threshold dose were independent of treatment and apparently also independent of season.

β carotene has also been shown to quench free radical formation *in vitro* (Fujimori & Tavla, 1966) and this may be another mechanism to explain its protective action. The findings of Mathews-Roth & Krinsky (1970b) suggest that in the organism *Sarcina lutea* carotenoid pigments act as preferred substrates for photosensitized oxidations.

At present an explanation cannot be offered for the reported protective effect of β carotene in EPP and its apparent failure in other photodermatoses where sensitivity to the same part of the spectrum exists. Further investigation to assess the role of singlet oxygen and free radical formation in porphyria, solar urticaria and actinic reticuloid is obviously essential.

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Retinoic acid in psoriasis: its value for topical therapy with and without corticosteroids

CLINICAL, HISTOLOGICAL AND ELECTRON MICROSCOPICAL STUDIES ON FORTY-FOUR HOSPITALIZED PATIENTS WITH EXTENSIVE PSORIASIS

CONSTANTIN E.ORFANOS,* HEINZ W.SCHMIDT, GUSTAV MAHRLE,
HEINZ GARTMANN AND WALTER F.LEVER

Department of Dermatology, University of Cologne, Germany and Department of Dermatology, Tufts University School of Medicine, Boston, Mass., U.S.A.

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SUMMARY

Topical application of retinoic acid (RA, 0·1 and 0·3% in a cream base) in psoriasis over 3 weeks showed that low therapeutic effect and frequent irritation limit considerably its usefulness in this disease. Of twenty treated patients, six had irritation warranting discontinuation of therapy, thirteen showed improvement, but none cleared completely. However, the therapeutic value was increased and the incidence of irritation was reduced if corticosteroids (CO) were added to RA. Of twenty-four patients treated with RA+CO, eighteen were improved and four cleared completely. Combined treatment seemed also to be more effective than CO alone in the same vehicle.

Light and electron microscopic studies after combined treatment showed reappearance of the keratohyaline, disappearance of parakeratosis and acanthosis and improvement of the dilated and fenestrated psoriatic capillaries. However, the number of tonofilaments was still decreased and some dermal changes were still present.

It seems that RA may act in psoriasis primarily by stimulating the synthesis of mucus and keratohyaline rather than by reducing the mitotic activity.

In 1962 Stüttgen and Beer reported independently that retinoic acid (RA, vitamin A acid) applied topically had a favourable effect in the treatment of various types of ichthyosis. These reports were followed by numerous clinical studies on the effect of the topical application of RA in patients with Darier's disease (Fulton *et al.*, 1968; Hesbacher, 1970) ichthyosis (Thomson & Milne, 1969; Mirrer & McGuire, 1970; Frost & Weinstein, 1969), psoriasis (Frost & Weinstein, 1969; Fry, Macdonald & McMinn, 1970; Fredricksson, 1971), acne (Kligman, Fulton & Plewig, 1969; Pedace & Stoughton, 1971; Bradford & Montes, 1970; Peachey & Connor, 1971), keratosis palmaris et plantaris (Heiss & Gross, 1970) and various hyperkeratotic and/or neoplastic epithelial disorders (Bradford & Montes, 1970; Schumacher & Stüttgen, 1971; Bollag & Ott, 1971).

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* Research Associate in Dermatology at Tufts University, Boston, Mass.

Requests for reprints: Professor Dr C.E.Orfanos, Univ.-Hautklinik, Köln-Lindenthal, Germany.

Although some of the reported data are contradictory, these studies showed that RA generally had a beneficial effect on most scaling dermatoses, particularly on those with increased epidermal proliferation, such as lamellar ichthyosis and psoriasis. The clinical use of RA seemed limited, however, since in a considerable number of cases its topical application caused erythema and exfoliation of the skin.

Since the irritant action of RA could be related to its capacity to labilize lysosomes (Kligman *et al.*, 1969), we assumed that the addition of corticosteroids to RA would decrease the incidence and the degree of irritation, possibly without affecting its value as a topical antipsoriatic agent.

In the present study we report the clinical and histopathological results obtained in forty-four psoriatics after topical RA treatment, with and without the addition of corticosteroids.

MATERIALS AND METHODS

Forty-four patients with extensive psoriasis of all types except pustular psoriasis were selected for this study. The patients were predominantly males (only five females), ranged in age between 10 and 73 years (average age 41 years), had bilateral lesions and had not been treated with corticosteroids or antimetabolites for at least 3 months prior to therapy with RA. All patients were hospitalized during the entire period of this study and concomitant internal diseases were excluded by standard laboratory tests.

The patients were assigned successively to four groups (I–IV), in order to compare two preparations of different RA concentration (0·1 and 0·3% RA in a cold cream base) with and without corticosteroids (CO, 0·25% fluor-methyl-dehydrocorticosterone (generic name Fluocortolone) and 0·25% fluor-methyl-dehydrocorticosterone capronate (generic name Fluocortolonecapronate):

Group I	Comparison: 0·1% RA cream vs cream base (placebo): ten patients	Twenty patients
Group II	Comparison: 0·1% RA cream vs 0·3% RA cream: ten patients	
Group III	Comparison: 0·1% RA+CO cream vs CO cream: twelve patients	Twenty-four patients
Group IV	Comparison: 0·3% RA+CO cream vs CO cream: twelve patients	

The clinical trials were designed as a double blind study (left-against-right). In each group the medication was applied to the psoriatic lesions on one side of the body and the control cream to the lesions on the other side (left-right treatment). All creams were issued in similar tubes, labelled with different colours and were applied once daily during the first week and—if no irritation appeared—twice daily for the next 2 weeks. In six patients, however, who were treated with creams containing RA alone, the treatment had to be discontinued as a result of considerable irritation. Dressings were not used in any of the patients.

Response to treatment was assessed daily by the physician and was recorded once a week in the patient's chart, especially designed for these studies. The clinical status was expressed according to the degrees of scaling and erythema as follows:

Improvement:	0	—none.
Improvement: NS	(±)	—not significant.
Improvement: moderate	+	—scaling improved, erythema unchanged.
Improvement: good	++	—scaling fully controlled, erythema improved.
Improvement: excellent	+++	—scaling and erythema fully controlled.

Histological examination was limited to lesions treated with RA + CO. Biopsies were taken after 1, 2 and/or 3 weeks of treatment from ten different patients (see Fig. 3). A total of sixteen biopsies were examined by light microscopy and eleven of them also by electron microscopy. Two biopsies obtained from non-treated psoriatic lesions served as controls for the electron microscopic studies. The biopsies were obtained under local anaesthesia and processed routinely for light microscopy. For electron microscopy the specimens were prefixed in a 6·5% solution of glutaraldehyde (buffered with phosphate buffer at pH 7·2) for 2 h, rinsed in the buffer solution, fixed in 1% osmium tetroxide for 1 h and embedded in Araldite. Thin sections were cut with a Reichert U2 ultramicrotome, were stained with uranyl acetate and/or lead citrate and were then examined with a Zeiss EM 9A electron microscope.

RESULTS

Clinical improvement in Groups I and II

In Group I topical treatment of psoriatic lesions with the 0·1% cream showed in six of ten patients significant improvement, whereas the lesions treated with the vehicle only showed no significant changes (Fig. 1). In Group II the 0·3% RA cream seemed to be more effective than the 0·1% cream, although the differences observed were not significant (Fig. 2). The overall response in the twenty patients of both groups treated with 0·1% RA cream was satisfactory (moderate to good) in thirteen patients, whereas in seven patients there was no significant improvement or none at all.

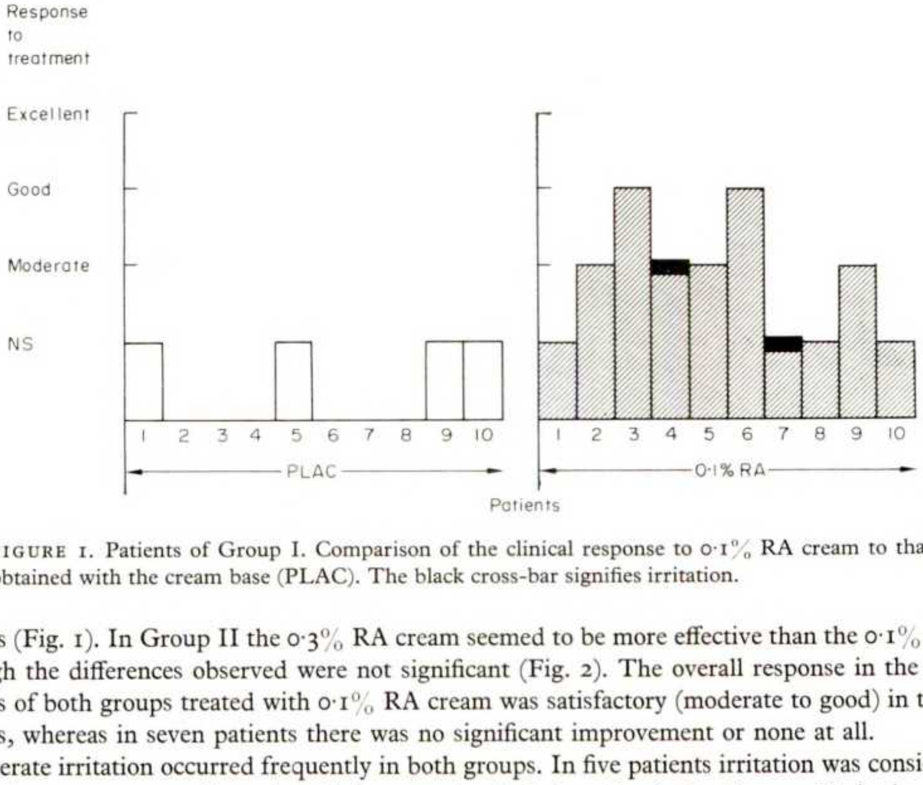

FIGURE 1. Patients of Group I. Comparison of the clinical response to 0·1% RA cream to that obtained with the cream base (PLAC). The black cross-bar signifies irritation.

Moderate irritation occurred frequently in both groups. In five patients irritation was considerable so that the treatment was discontinued after 2 weeks. A sixth patient had widespread irritation after 3 days and refused further treatment. The irritation imitated subacute dermatitis and was clinically more pronounced in patients using the RA cream with the higher concentration (0·3%). Visible irritation was limited to the treated lesions and the immediate surrounding skin. Itching was experienced by four patients, three of whom had visible irritation.

In all thirteen patients showing improvement, whether with or without irritation, the same consecutive changes were seen in the lesions during treatment with RA creams: Increased scaling→

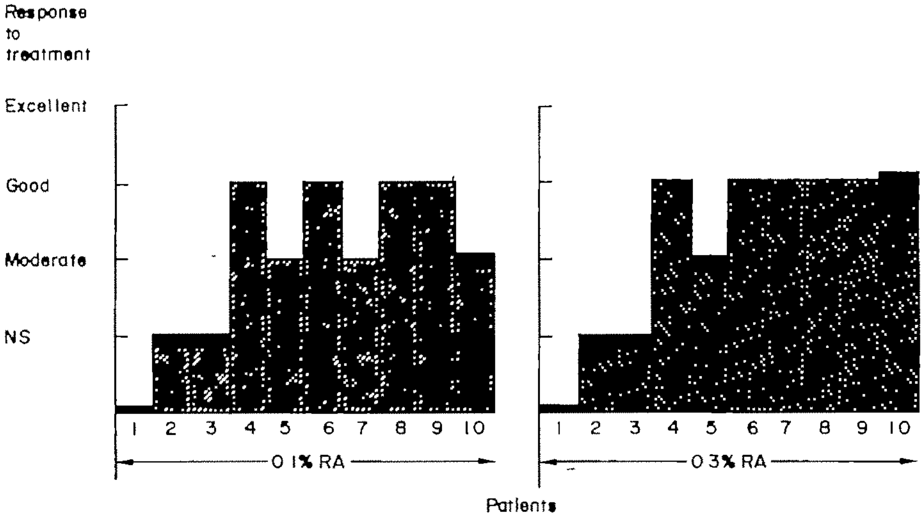

FIGURE 2. Patients of Group II. Comparison of the clinical response to 0·3% RA cream to that obtained with 0·1% RA cream. The black cross-bar signifies irritation.

reduced scaling→reduced erythema→reduced infiltration. However, none of the patients in these two groups had cleared completely after 3 weeks.

Clinical improvement in Groups III and IV

The following results were obtained in the twenty-four patients treated with combined RA+CO creams (Fig. 3):

(a) Comparison of the combined RA+CO creams with the control cream containing CO alone

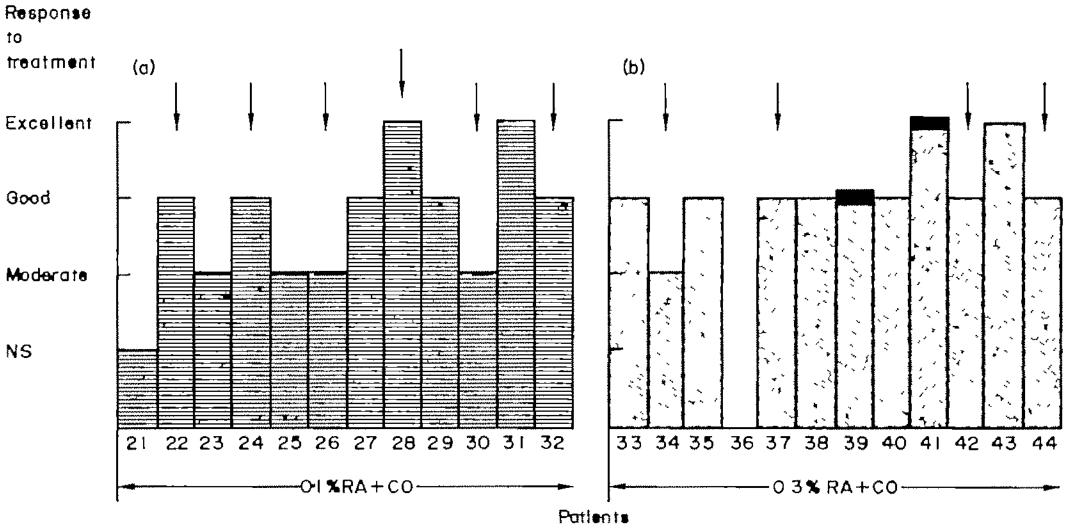

FIGURE 3. Patients of (a) Group III and (b) Group IV. Comparison of the clinical response to the combined RA+CO creams. Arrows, patients from whom biopsies were taken. The black cross-bar signifies irritation.

revealed a better response to the combined treatment in twelve patients, no difference in eight patients, and a better response to corticosteroids in three patients. One patient was not co-operative and was not evaluated (No. 36, Fig. 3).

(b) Comparison of the patients in Groups III and IV showed slight but not significant differences between the two combined creams in regard to their therapeutic value (Fig. 3).

(c) The average clinical improvement achieved with the two combined preparations was good (Fig. 4), better than the average response of the patients treated with RA only (Compare Figs. 2 and 3). In Groups III and IV, four patients out of twenty-four cleared completely in 3 weeks, whereas no full recovery was seen in any of the patients in Groups I and II in the same period of time.

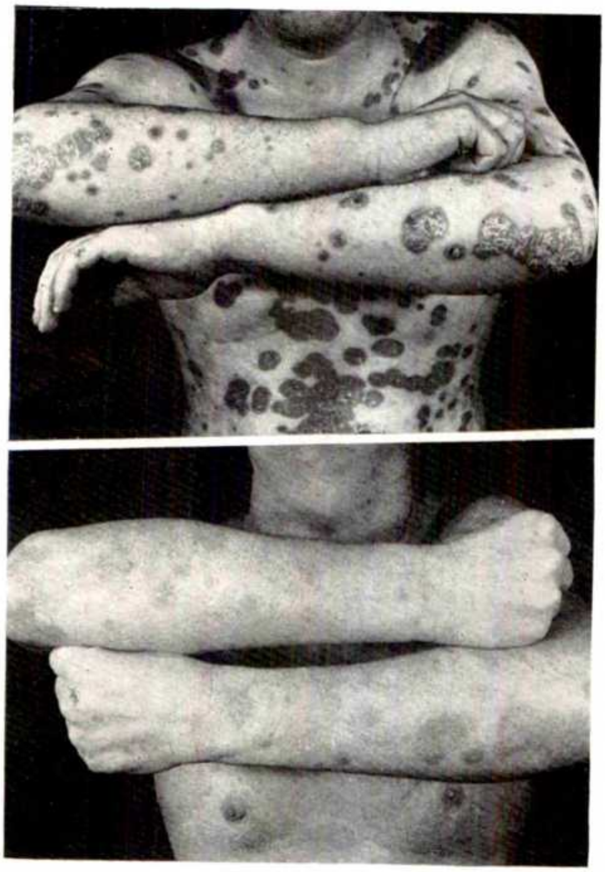

FIGURE 4. Extensive psoriasis (pat. no. 37) before (upper photograph) and after (lower photograph) 2½ weeks of combined RA + CO treatment (right side of patient) vs treatment with corticosteroids only (left side).

(d) The addition of fluorinated corticosteroids to RA suppressed irritation completely in all patients of Group III and decreased considerably the frequency and the degree of irritation in the patients of Group IV (Fig. 5). None of these patients were unable or unwilling to tolerate the RA + CO treatment, contrary to six of twenty patients treated with RA only.

The sequence of changes in the lesions and the mode of healing seemed to be the same after RA cream with and without corticosteroids. In all patients showing improvement this was clinically evident after 8–10 days, whereas during the last days of treatment less improvement was seen. Non-infiltrated lesions of psoriasis, such as guttate psoriasis, responded better to treatment than papulosquamous lesions or lesions of generalized erythrodermic psoriasis. The age of the patients had seemingly no influence on the degree of healing. However, a 10-year-old boy showed an excellent response to treatment within a few days. No internal complications were observed after RA or RA + CO treatment.

Light microscopic examination revealed, as a rule, after 1 week's treatment with combined RA + CO creams that numerous ketatohyaline granules were present in a few areas, although parakeratosis without a stratum granulosum and acanthosis still predominated (Fig. 6). After 2 weeks of treatment the number and the size of the granules had increased and distinct hypergranulosis was frequently observed. Furthermore, the parakeratosis had disappeared in most cases, but the epidermis still had a psoriatic appearance because of persistent acanthosis. After 3 weeks the epidermis often appeared normal or nearly normal, whereas dermal changes, especially papillary oedema and numerous dilated vessels, were still regularly present.

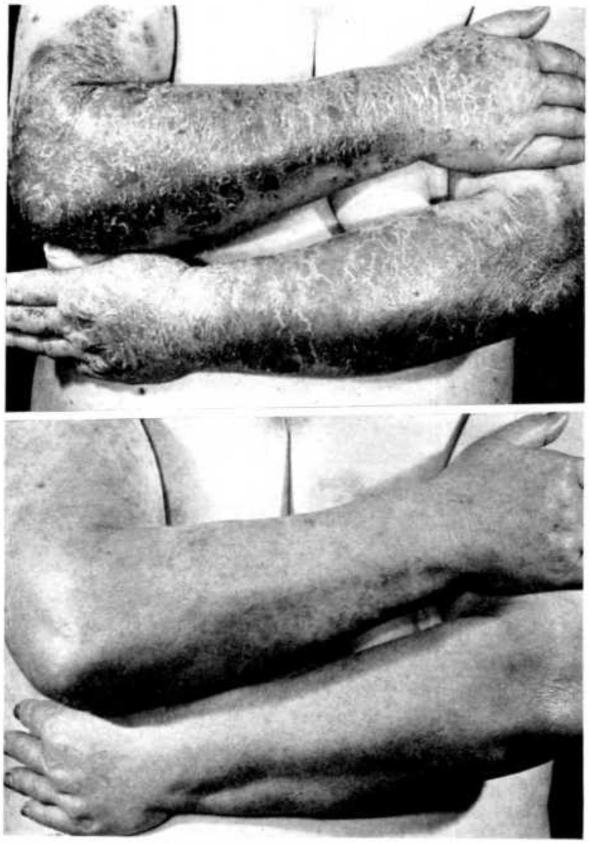

FIGURE 5. Good response both to RA + CO (right side of patient) and to CO creams (left side). On the right arm slight irritation due to RA is seen (pat. no. 39).

Additionally, in some specimens obtained after 2 or 3 weeks, dermatitis-like features were observed, such as spongiotic vacuoles in the epidermis and diapedesis and accumulation of red cells in the upper dermis, obviously the result of irritation from RA (Fig. 7).

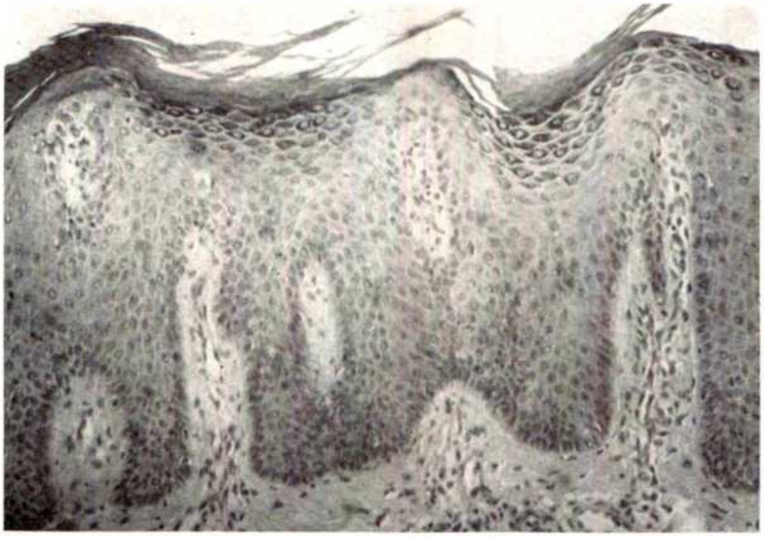

FIGURE 6. Psoriatic epidermis after 1 week's treatment with RA + CO. Focal hypergranulosis is seen whereas parakeratosis and acanthosis are still present (× 75).

Electron microscopic examination revealed findings analogous to the light microscopic data:

(a) After 1 week's treatment numerous large vacuoles, 0·4–1·6 μm in diameter, were observed in several psoriatic keratinocytes, containing a fine granular material of low electron density. In addition, considerable amounts of amorphous material of medium electron density were found in the intercellular spaces of the upper malpighian and the granular layers (Fig. 8). These findings were less pronounced after 2 weeks and nearly absent after 3 weeks. None of our control specimens showed these changes, although recently similar vacuoles have been described (Nagy-Vezekenyi & Zs-Nagy, 1971).

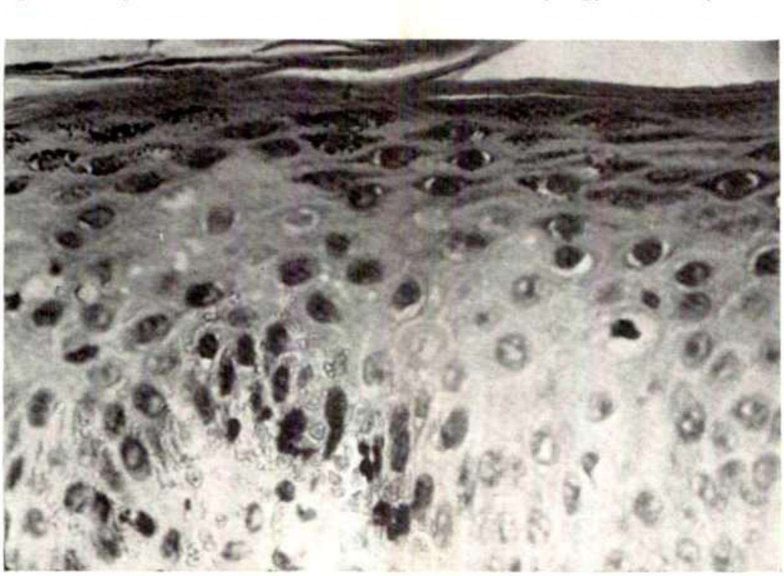

FIGURE 7. Treated psoriatic epidermis (2 weeks after RA + CO) showing normal appearing granular layer, mild intraepidermal invasion of leukocytes and accumulation of erythrocytes in the lower epidermis (RA irritation) (× 260).

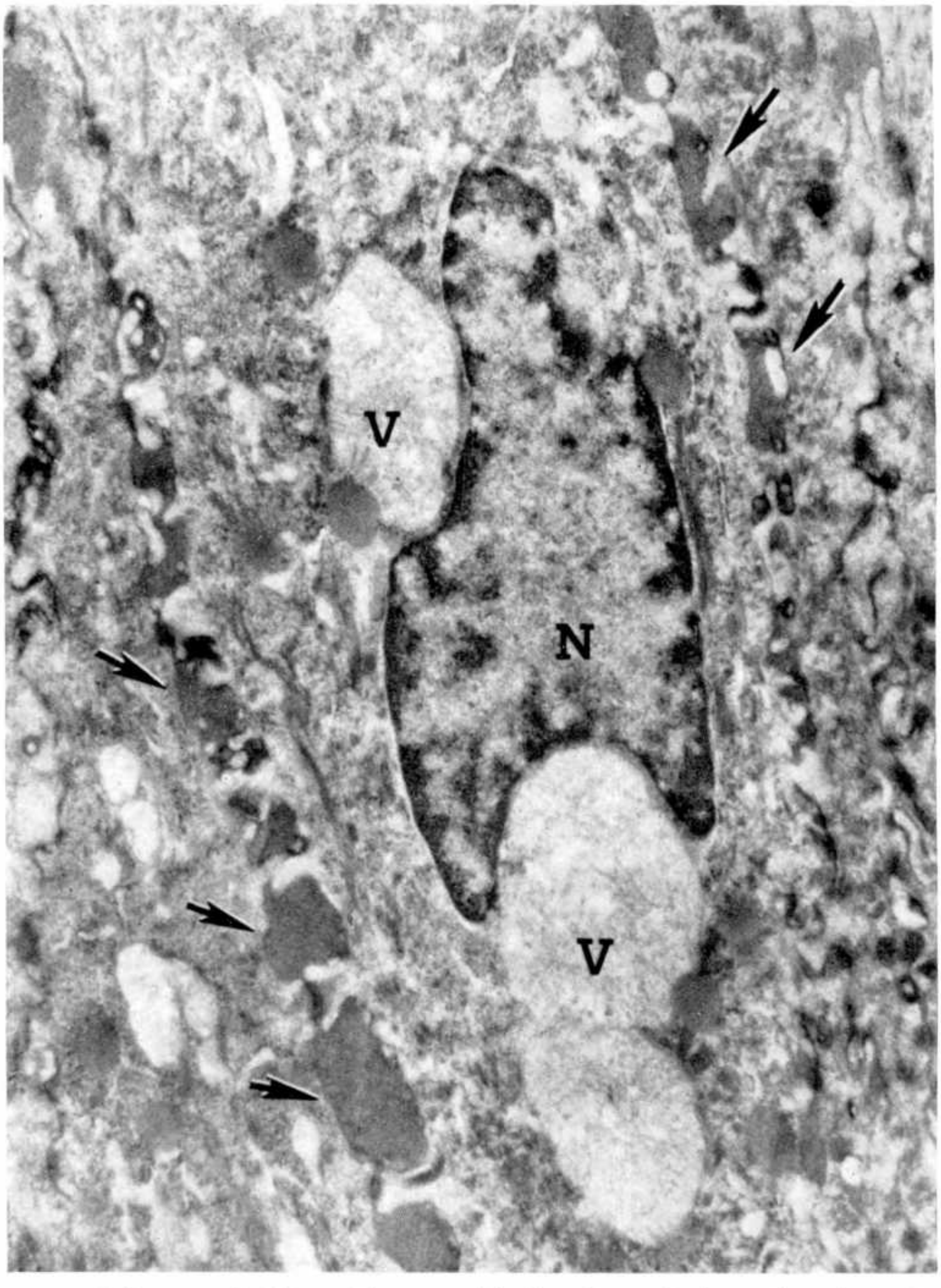

FIGURE 8. *Large vacuoles* (V) containing a material of low electron density are located near the nucleus (N) of a malpighian keratinocyte. Amorphous mucus-like material fills the intercellular spaces (arrows). Specimen after 1 week of RA + CO treatment (× 21,000).

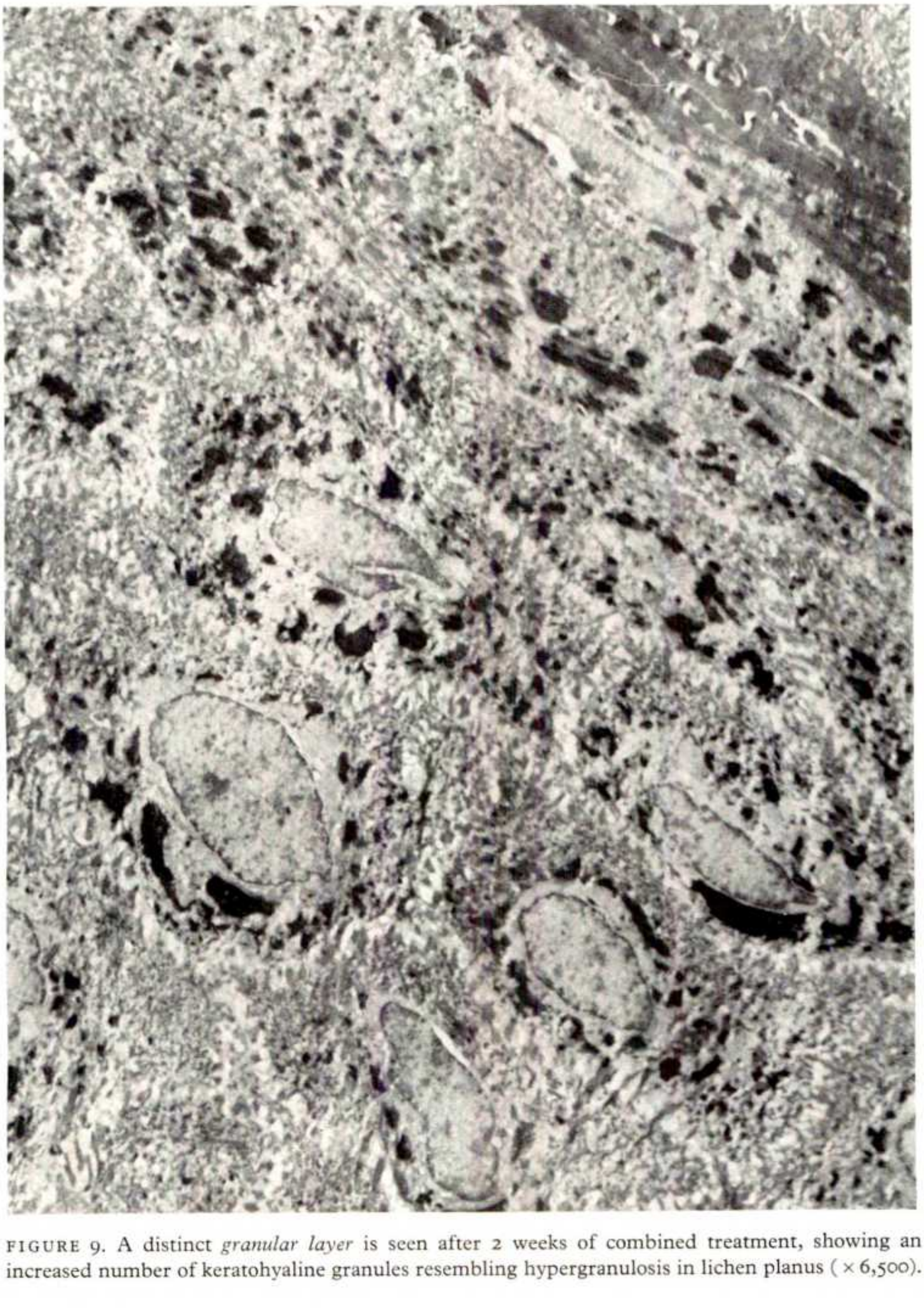

FIGURE 9. A distinct *granular layer* is seen after 2 weeks of combined treatment, showing an increased number of keratohyaline granules resembling hypergranulosis in lichen planus (× 6,500).

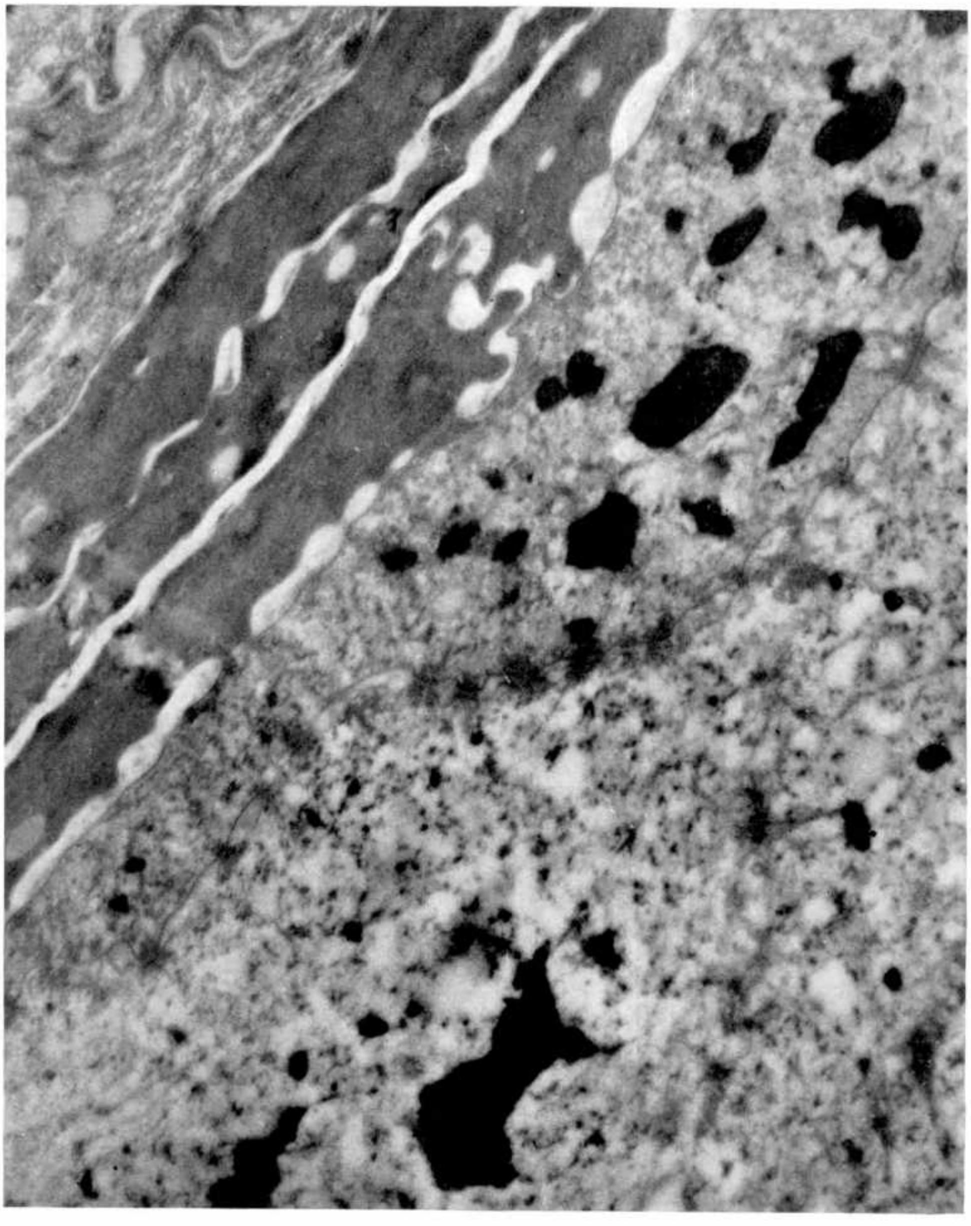

FIGURE 10. Only *few tonofilaments* are present in the oedematous cytoplasm of a granular cell in spite of the presence of numerous large keratohyaline granules. Parakeratosis has disappeared and an essentially normal horny layer is seen. Specimen after 3 weeks of combined treatment (× 19,000).

(b) Keratohyaline granules were present in most specimens after 1 week's treatment, but were small and scarce in most examined areas. Their number and size increased after 2 and 3 weeks of treatment, and a distinct stratum granulosum was then found regularly. The granular cells after 3 weeks of treatment were oedematous and the numerous keratohyaline granules within them appeared irregular in size and shape (Figs. 9 and 10).

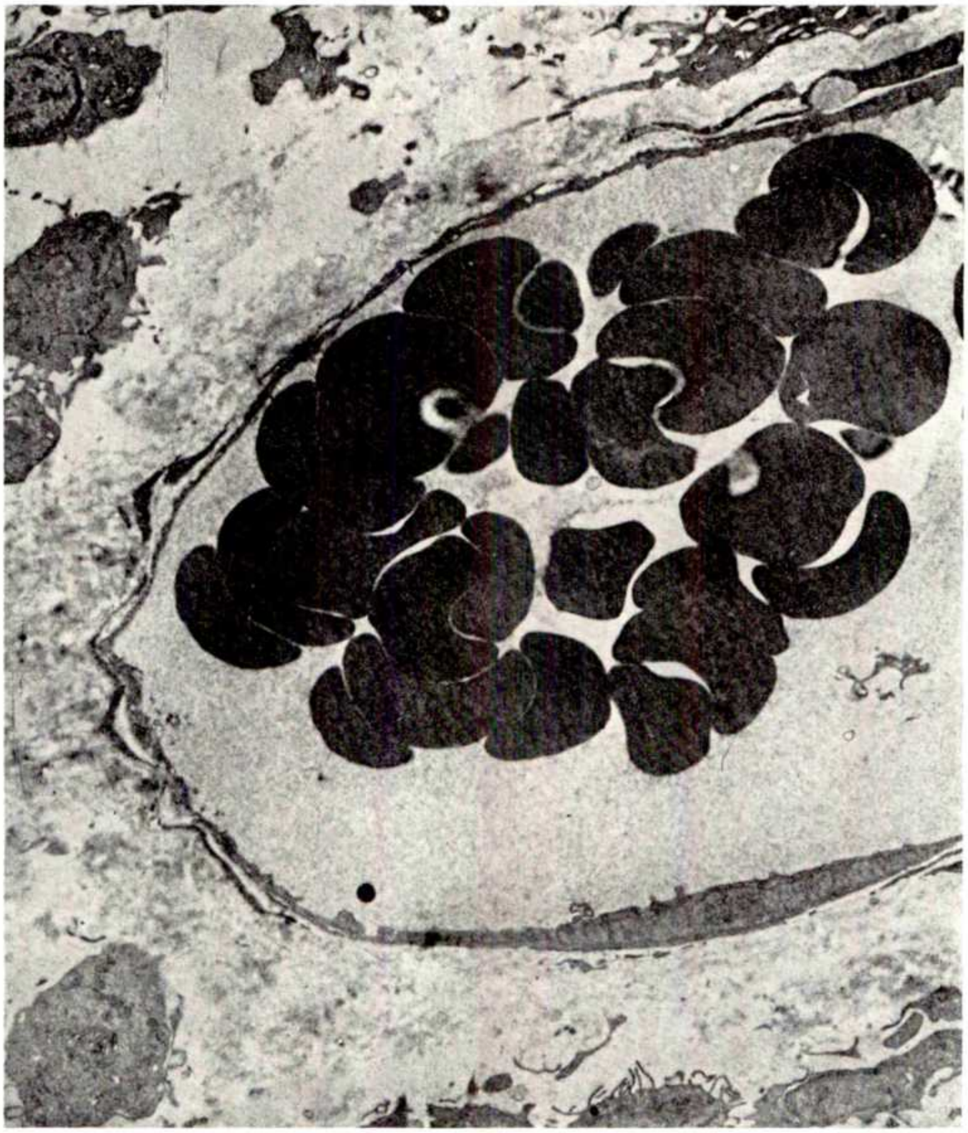

FIGURE 11. *Capillary in an untreated psoriatic lesion* is dilated, filled with erythrocytes (stasis) and shows extreme thinning of the vessel wall (× 4,950).

(c) Tonofilaments were scarce or nearly absent after 1 week's treatment and were still reduced in number after 2 and 3 weeks of treatment (Fig. 10).

(d) Parakeratosis was markedly improved after 2 weeks of treatment and in most cases was absent after 3 weeks, having been replaced by a normal or even thickened stratum corneum (Fig. 10).

(e) The capillaries of the papillary layer in the untreated lesions and in those treated for 1 week were numerous and greatly dilated and showed extreme thinning of their walls (Fig. 11). In several capillaries, located a considerable distance from the epidermis, endothelial gaps were seen (Fig. 12), resulting in exocytosis in some areas. Extensive discontinuities of the walls, resembling incomplete capillary formation, was seen in the dermal papillae close to the epidermis. After 3 weeks there still were numerous dilated, thin-walled capillaries; however, the diameter of their lumina seemed reduced and no leakages were observed.

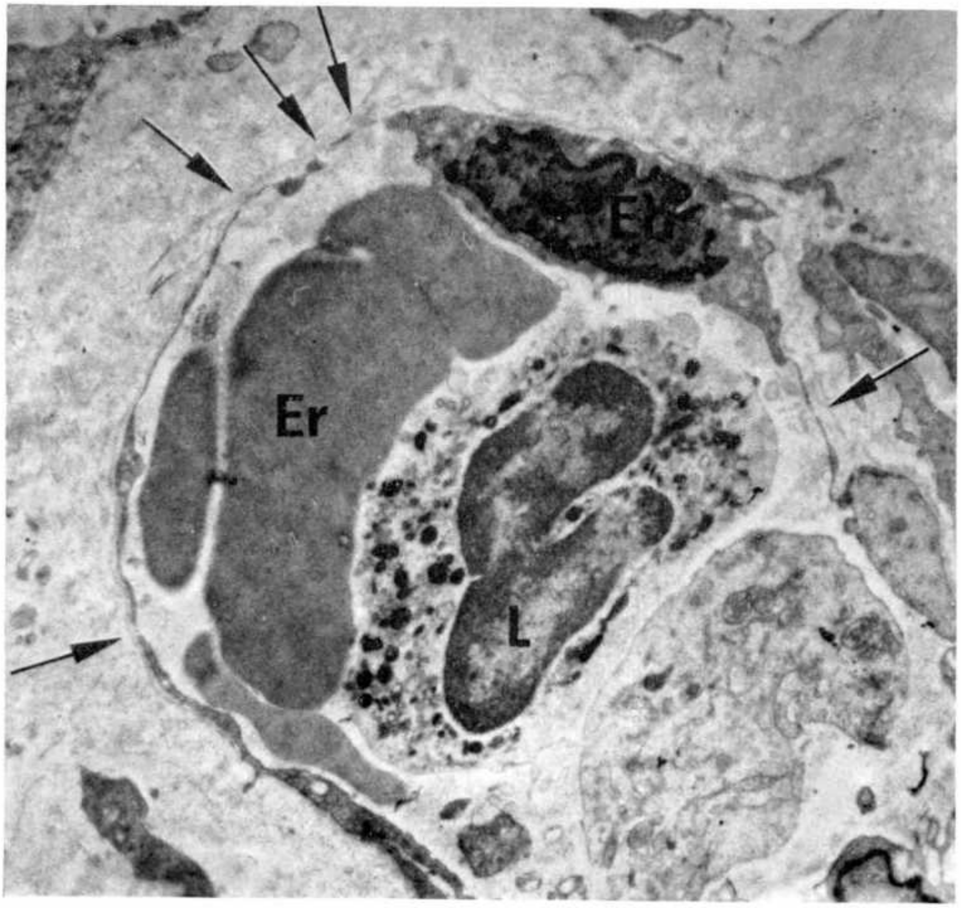

FIGURE 12. *Capillary in a treated psoriatic lesion* (1 week after RA+CO application) still shows dilatation, thinning and gaps within the vessel wall (arrows). L, leukocyte; E, erythrocyte; En, endothelial cell (× 11,350).

(f) In addition to neutrophils and histiocytes, cells with highly indented (cerebriform) nuclei were seen in three specimens within the psoriatic epidermis after 1 or 2 weeks of treatment (Fig. 13). These cells were similar to those described previously in dermal lymphomatous infiltrates (Flaxman, Zelazyn & Scott, 1971).

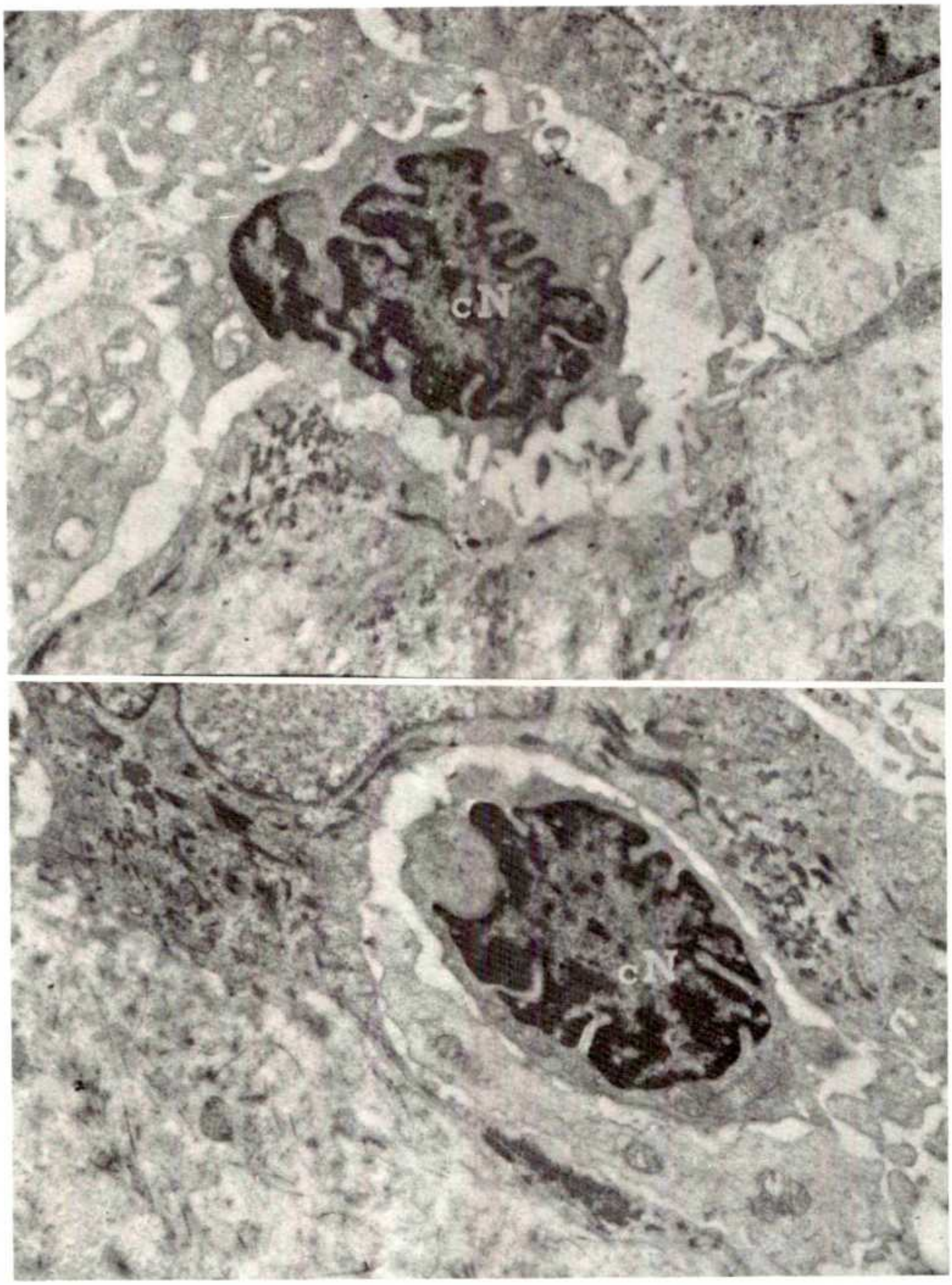

FIGURE 13. Cells with *cerebriform nuclei* (cN) are seen within the psoriatic epidermis. Specimen was obtained after 17 days of combined treatment (× 11,350).

The impression was gained that the concentration of RA used in the RA creams had no significant influence on the light and electron microscopic findings.

DISCUSSION

Clinical efficacy of the RA creams

The observations reported here confirm the findings of other authors that RA has some beneficial effect when applied topically in psoriasis (Frost & Weinstein, 1969, Fry *et al.*, 1971; Fredriksson, 1971). Furthermore, the results were similar to those obtained after the systemic administration of this drug (Orfanos *et al.*, 1972). However, we consider the antipsoriatic effect of RA as insufficient to recommend its use as a therapeutic modality in psoriasis. Its value is further limited by the frequent occurrence of irritation which proved to be a considerable disadvantage in psoriasis, just as it has been in acne (Peachey & Connor, 1971). Because of RA-irritations in six of twenty patients the treatment was discontinued, since irritation from topical agents may cause new lesions in psoriatics (Steigleder & Orfanos, 1967), acting as an iatrogenic Koebner phenomenon.

Addition of fluorinated corticosteroids to the RA clearly enhanced the therapeutic effect. A major advantage of the combined treatment was that irritation was largely avoided and the treatment could be successfully carried out for the entire period in all patients. This suggests that visible irritation of the skin is not required for the RA to be therapeutically effective. The average response to the combined treatment was also clinically better than the corticosteroids alone in the same vehicle. Thus, the two drugs seem to enhance their antipsoriatic effect rather than to neutralize each other. Similarly, better results have been reported by Whittle *et al.* (1961) with combined treatment with ointments containing vitamin A and triamcinolone.

Nevertheless, it seems that long-term trials with RA + CO are necessary in psoriasis since only four of twenty-four patients (i.e. 17%) had cleared completely after 3 weeks. It has to be determined whether full recovery can be obtained regularly and whether the tendency of psoriatic lesions to relapse after this therapy is as great as it is after the topical application of corticosteroids alone. A reduction in the concentration of corticosteroids used here is another important goal for future investigations. At present we believe that the combined RA + CO application is an acceptable treatment in psoriasis and warrants further study.

Tissue changes and mode of action of RA

The mechanism of the antipsoriatic action of RA still is a matter of controversy and speculation. Its topical application has been shown to stimulate the mitotic and metabolic activity of normal keratinocytes in guinea-pigs (Wolff, Christophers & Braun-Falco, 1970) and in humans (Plewig, Wolff & Braun-Falco, 1971) whereas in psoriatic epidermis it reduces the mitotic count (Fry *et al.*, 1970). Furthermore, it induces mucous secretion in the neoplastic keratinocytes of rabbit keratoacanthoma (Prutkin, 1967).

This study revealed that after 2–3 weeks of RA + CO treatment increased amounts of keratohyaline granules had appeared and orthokeratosis was largely restored in psoriatic epidermis. Moreover, the extremely dilated and fenestrated capillaries which were regularly present in non-pustular psoriasis, similar to those described in pustular psoriasis (Braverman, Cohen & O'Keefe, 1972), were also markedly improved.

The reappearance of keratohyaline granules in psoriatic epidermis after treatment with RA seems well established (Fry *et al.*, 1970; Fredriksson, 1971) and is confirmed here by electron microscopic data. Whether this is caused by a primary granulogenic effect of RA or is secondary to the reduction of mitosis is not clear. However, since the present study has shown that the newly formed granular cells have reduced amounts of tonofilaments in their cytoplasm, and consequently do not represent normal fully differentiated keratinocytes, a direct granulogenic effect of RA seems more likely. Ultrastructurally, the granular cells after RA application in psoriasis resemble the granular cells in lichen planus (Orfanos, 1972).

The presence of amorphous intercellular material in psoriatic epidermis 1 week after RA application may be ascribed to the stimulating influence of RA on the synthesis of mucus (Wolff *et al.*, 1970; Prutkin, 1967), similar to the effect of vitamin A on embryonic chicken epidermis (Jackson & Fell, 1963). The fact that this material reacts positively with periodic acid–silver methenamine (Wolff *et al.*, 1970) (PASM-reaction) indicates the presence of glycoproteins. In the non-keratinized layers of untreated psoriatic epidermis the PASM-reaction was shown to be negative (Orfanos, Schaunburg-Lever & Lever, 1972) and previous investigations on psoriatic epidermis treated with corticosteroids alone do not mention the presence of intercellular mucus-like material (Kalkoff & Berger, 1965). In conclusion, it seems likely to us that RA has an initial mucogenic and then a granulogenic effect in psoriasis, which may be major reasons for its value as antipsoriatic agent.

In regard to the question as to how RA reduces the mitotic activity in psoriatic epidermis no clear answer can be given. However, it is possible that the synthesis of mucus may cause an increase of the deficient glycoprotein-rich surface coats in psoriasis and thus restore the suggested loss of 'contact inhibition of growth' (Orfanos *et al.*, 1972).

Finally, the present study confirms that cells with cerebriform nuclei occur in skin diseases other than mycosis fungoides and Sezary's syndrome. In psoriasis these cells are not limited to the dermal infiltrate as previously shown (Flaxman *et al.*, 1971), but occur also within the epidermis, presumably as a result of RA-irritation.

ACKNOWLEDGMENTS

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Case Reports

Generalized pustular psoriasis treated with Dapsone

A.L.MACMILLAN AND R.H.CHAMPION

Addenbrooke's Hospital, Hills Road, Cambridge

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SUMMARY

Dapsone (diaminodiphenylsulphone) combined with triamcinolone, has effectively suppressed very severe generalized pustular psoriasis in one patient for over 18 months.

Generalized pustular psoriasis (GPP) runs a very variable course, often showing spontaneous remissions and is usually best left without active treatment. However, the type which follows acral pustular psoriasis or acrodermatitis continua of Hallopeau seems to have a very bad prognosis. Of eleven such patients reported by Ryan & Baker (1971), seven died, one was severely disabled, one had recurrent exacerbations, one had persistent acral psoriasis and only one was in remission. We report successful suppression for over 18 months of generalized pustular psoriasis (GPP) of the type following previous acral psoriasis (Baker & Ryan, 1968) which is also called generalized acrodermatitis continua pustulosa of Hallopeau.

CASE REPORT

A male university lecturer born in 1923 was fit and without skin disease until 1960 when he developed localized acral pustular psoriasis (Fig. 1). This spread over several months to involve groin, scalp, elbows, forearms and feet but largely cleared in 1962 leaving only knees, elbows and one heel involved by localized pustular psoriasis. He had frequent progressively more severe exacerbations with severe systemic disturbance producing increasing persisting disability. For 7 years active treatments were avoided. Treatments which were used included bed rest with sedation and bland topical applications and later topical Exolan (triacetoxyanthracene 1%), topical fluorinated corticosteroids, topical methotrexate, oral chloroquin, oral erythromycin and beta-ray therapy. In 1969 systemic methotrexate as daily oral doses, weekly oral doses and later weekly intravenous doses produced side-effects without affecting the pustular psoriasis. Early in 1970 he had transient dramatic apparent benefit from Nystatin ointment. In March 1970 he developed a severe exacerbation with fever and pustulation of limbs, head and neck. Despite conservative treatment his condition deteriorated and pustulation and erythema became generalized. Azothioprine was given for several weeks without effect and stopped when he developed severe jaundice. By June 1970 he was weak, emaciated, had difficulty in using his hands to feed himself and had fresh crops of generalized pustulation every 1 or 2 days. He showed an intermittent or continuous fever up to 40° C, neutrophil leucocytosis of 21,000/mm^3 and an erythrocyte sedimentation rate of 116 mm/h. Repeated swabs from the pustules and repeated blood cultures were sterile. He was expected to die in days or weeks. He was then treated with triamcinolone 72 mg/day which produced dramatic improvement. When the dose had been reduced over 3 months to 15 mg/day, generalized pustulation and malaise recurred. Dapsone (diaminodiphenylsulphone) initially 300 mg/day was given and the pustules and malaise cleared. Since October 1970 on dapsone 200–125 mg/day and triamcinolone 12–8 mg/day, his skin has shown only mild redness and occasional scant pustules, his nails have regrown, he feels increasingly well, he has regained full use of his hands and has almost returned to full work. Mild relapses have been precipitated by reduction in the dose of triamcinolone and of dapsone. Twice in the last few months, a reduction of the dose of dapsone from 125 mg/day to 125 mg and 100 mg on alternate days has produced increasing redness of the skin and occasional pustules within 3 days of reducing the dose. His haemoglobin level is maintained at 13 g/100 ml blood with a reticulocyte count of 6%.

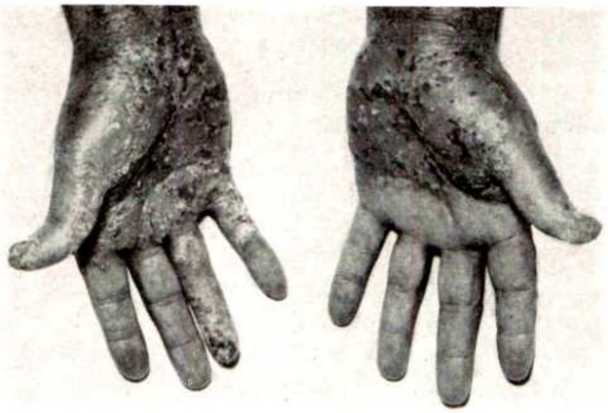

FIGURE 1. Acrodermatitis continua of hands in 1966.

DISCUSSION

Sulphones and sulphapyridine are effective treatment for a remarkable variety of dermatoses, including some with apparently sterile pustulation (Lorincz & Pearson, 1962). Sulphapyridine was introduced into dermatology by Sulzberger (1939a, 1939b). He used it to produce dramatic remission of localized acrodermatitis continua pustulosa of Hallopeau in a nurse, after many ineffective treatments including sulphanilamide. Similar cases were reported by Lever (1944) and Ashurst (1956). Curtis & Netherton (1942) reported a case which they said responded to sulphathiazole. Schoch & McCuiston (1955) gave salicylazosulphapyridine to patients with a wide variety of dermatoses and claimed improvement of one of four patients with 'pustular psoriasis'.

These drugs also suppress some generalized apparently sterile pustular dermatoses. Sneddon & Wilkinson (1956) suppressed subcorneal pustular dermatosis in most of their patients with sulphapyridine or dapsone. Hellier (1956) described a patient with a diagnostic label of generalized pustular bacterid who showed the clinical features of subcorneal pustular dermatosis but histologically there was spongiosis about the pustule which suggests a diagnosis of GPP. The rash was suppressed by sulphapyridine and later by dapsone with fewer side-effects. In 1944 Hall described a man with impetigo herpetiformis suppressed by sulphapyridine. From the clinical description (there was no biopsy) the patient probably had either annular GPP (Degos, Civatte & Arrouy, 1966) or subcorneal pustular dermatosis.

We believe that continued suppression of this patient's GPP following acral psoriasis is due to the combined effects of dapsone and triancinolone and that after 9 years of inexorable deterioration, this therapy has proved life-saving. No general conclusions can be drawn from a single case, but the dismal prognosis in this type of GPP suggests that the use of dapsone or sulphapyridine, perhaps in combination with triamcinolone, deserves further trial in the most severe cases although we entirely agree with Ryan & Baker (1969, 1971) that the majority of cases are best left without any active treatment.

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Ataxia telangiectasia

CASE REPORT OF A BENIGN VARIANT WITH TELANGIECTASIA RECURRENT INFECTION AND LOW IgA

L. STANKLER AND FIONA M. BENNETT

Departments of Dermatology and Ophthalmology, Aberdeen Royal Infirmary, Aberdeen

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SUMMARY

A healthy man aged 34 was found by chance to present several features suggestive of ataxia telangiectasia (AT). The association between telangiectasia, recurrent infection and deficiency in IgA is discussed in relation to the findings in this patient.

A group of diseases has been described which have in common a photosensitive telangiectasia (Landau *et al.*, 1966). Two of them, Bloom's syndrome (Landau *et al.*, 1966) and AT (Fireman, Boesman & Girlin, 1964; Reed, Epstein & Boden, 1966; Ammann, Roth & Hong, 1970) have an immunological defect. The latter condition is (1) an autosomal recessive disorder (Boder & Sedgwick, 1963) with (2) progressive cerebellar ataxia, (3) cutaneous and mucosal telangiectasia, (4) deficient or absent thymus, (5) immunoglobulin deficiencies, (6) predisposition to infection and to (7) malignancy, and (8) abnormal ocular movements (Smith & Cogan 1959). The findings in the patient to be described resemble most closely those seen in AT.

CASE REPORT

A man aged 34 was seen in 1971 for three nodules which had been noticed 6 months previously and were subsequently excised and found to be histiocytomata. At the time of examination telangiectases were noticed on the left arm. Questioning elicited the following history. At the age of 3 he fell and dislocated the left shoulder. In the process of reducing the dislocation the doctor squeezed the upper arm and produced 'spots' at the sites of pressure. Since that time throughout childhood and early manhood any injury to the left hand, no matter trivial, was followed by infection at the site, swelling of the arm and painful nodes behind the left elbow and in the left axilla. Each episode of infection was followed by the progressive appearance of more 'spots' until most of the arm and adjoining region of the back were involved. The patient subsequently noticed that the skin of the left shoulder was more difficult to pinch than that of the right. The story was corroborated from his case records in which there was mention of an admission to hospital in 1957, at the age of 19, for treatment of cellulitis, lymphangitis and lymphadenitis of the left arm following a cycling accident in which he sustained minor cuts to the left hand. The infection settled rapidly with penicillin injections. In

March 1970 he attended the Eye Department with a 6 months' history of double vision and was found to be suffering from a vertical diplopia with an alternating divergence, the cause for which could not be ascertained. An X-ray of the skull was normal and a WR was negative. In June of that year he was operated on for a squint. His ophthalmic history was complicated by lime burns of both conjunctivae and corneae in July 1971 which were followed by diplopia and conjunctivitis. He subsequently had repeated episodes of 'conjuctivitis' and on several occasions swabs of the eyes grew no organisms. A second operation for squint was carried out in March 1972. Review of his ophthalmic state in May 1972 revealed nystagmoid jerks in the mid-position of right and left versions and difficulty in turning the eyes on a horizontal plane. There was defective convergence of the right eye for close work and congestion of the conjunctival vessels. Neurological examination was otherwise normal. The family history was non-contributory and his parents were not related. On examination he was found to have widespread telangiectasia of the left arm, extending to the contiguous area of the back. The skin of the upper arm felt sclerodermatous. Telangiectases were present on the soft palate. Total white cell and differential counts were normal. His immunoglobulin levels were: IgG 1400, 1100 (700–1800), IgA 40, 44 (90–450) IgM 94, 92 (60–280). A biopsy of skin showed histological features consistent with AT.

DISCUSSION

AT is an unusual autosomal recessive disorder characterized by a progressive cerebellar ataxia (Louis-Bar, 1941) and the gradual symmetrical appearance of telangiectasia over light-exposed areas, antecubital fossae, buccal mucosa and bulbar conjunctivae. Dry skin, follicular keratoses, solar keratoses, basal cell epitheliomata, atrophic and sclerodermatous changes may occur, as may eczema of seborrhoeic, atopic or nummular type. Diffuse greying of the scalp hair, often at an early age, and extensive warts are common findings (Reed *et al.*, 1966). Thymic deficiency, IgA and IgE deficiencies may predispose to sino-pulmonary infections, impetigo and malignancy—usually lymphoreticular.

This patient shares the following features with AT; telangiectasia of the skin and mucous membranes, repeated cutaneous infection, abnormal ocular movements and deficiency in IgA. It may be an unusual benign variant, as there is no evidence of ataxia and both the telangiectasia and recurrent infection involve one limb only. The cause of the vascular changes in AT is not known. It has a predilection for exposed sites (Reed *et al.*, 1966) and may result from a mesenchymal defect (Peterson, Kelley & Good, 1964). The respiratory infection has been attributed to the increased mucosal vascularity (Bowden, Danis & Sommers, 1963). Observations in the present patient suggest that the vascular changes follow infection and this finding may explain the presence of abnormal capillaries in areas of bronchiectasis (Dunn *et al.*, 1964) which is a common cause of death in AT. It is of interest that the telangiectasia in rosacea (Sneddon, 1966) and essential progressive telangiectasia (Shelley, 1971) clears with prolonged tetracycline therapy. Recurrent sino-pulmonary infection has been attributed to deficiency in IgA (Fireman *et al.*, 1964) and IgE (Ammann *et al.*, 1970). The localized nature of the infection in this patient suggests a localized susceptibility.

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Comment

LIPOPROTEINS IN RELATION TO SKIN DISEASE

The classical work of Fredrickson, Levy & Lees (1967) demonstrated that abnormally high concentrations of serum lipoproteins can occur in xanthomatosis, and that upon application of a suitable diet and drug therapy, the xanthomata often disappear.

Ultracentrifugation, and electrophoresis, using paper, cellulose acetate or agarose gel, have shown the presence of four groups or families, of serum lipoproteins, the chylomicrons, pre-β-lipoproteins, β-lipoproteins and α-lipoproteins (de Lalla & Gofman, 1954; Lees & Hatch, 1963; Charman & Landowne, 1967; Noble, 1968). It is the first three groups which have been correlated with xanthomatosis by Fredrickson, but before dealing with the classification it would perhaps be of some help to consider the composition and function of the lipoproteins.

The lipoproteins can be considered to be protein aggregates associated with varying amounts of lipids. The protein moiety within each group consists of a polypeptide chain to which is attached carbohydrate by covalent linkages; this is the apoprotein which is then capable of binding lipid. The lipids involved are glycerides, cholesterol and its esters, and phospholipids. Little is known about the function of the latter except that they appear to enter into fundamental enzyme reactions (Scanu, 1971). Fatty acids are bound to albumin and will not be dealt with here.

Since lipids are relatively light materials, the resulting particles of lipid and protein are less dense than other proteins, and may be separated from them by careful centrifugation. The classification of serum into the Fredrickson Types by the simple observation of the serum after storing overnight at 4°C (Havel, 1969) also depends upon the density of the lipoproteins.

Furthermore, since the lipids are insoluble in water they impart a turbidity to the serum, the degree of which may be measured in a micronephelometer and correlated with the concentration of the lipoproteins (Stone & Thorp, 1966).

The chylomicrons are the largest and least dense of the lipoproteins and are synthesized in the intestinal mucosa from dietary triglycerides having chain lengths of twelve carbon atoms or more. They are then carried to adipose and other tissues such as liver, heart, spleen, and skeletal muscle, where lipoprotein lipases hydrolyse the triglyceride components (either at the capillary surfaces or immediately on entering the tissue cells), giving glycerol and fatty acids. Consequently the chylomicrons collapse and the fatty acids are either resynthesized into other glycerides, and phospholipids, or cholesterol esters, or oxidized to carbon dioxide and ketone bodies.

In contrast to the chylomicrons, the pre-β-lipoprotein aggregates are smaller and more dense. They are rich in endogenous triglycerides, most of which are of hepatic origin (Windmueller & Levy, 1967), although recently Ockner & Jones (1970) have demonstrated intestinal production. Clearly the concentration of pre-β-lipoprotein in the plasma is partly controlled by the availability of fatty acids and glycerol, both of which can be obtained from dietary carbohydrate (Nikkilä, 1969).

The β-lipoprotein aggregates are smaller and more dense again than pre-β-lipoprotein. They are also richer in cholesterol and contain more phospholipid. The α-lipoprotein aggregates are yet smaller and more dense, and contain mainly cholesterol and phospholipid with a little triglyceride.

There is a divergence of opinion as to the site of synthesis of β and α-lipoprotein. Until recently it was thought that both were synthesized only in the liver, but Windmueller & Levy (1968) found them in mesenteric lymph. It was thought that there was no connection between the synthesis of pre-β, β, and α-lipoprotein, and that each was produced independently. However, Gobbo *et al.* (1972) have confirmed the recent suggestion by Bilheimer, Eisenberg & Levy (1971), that in fact β-lipoprotein is synthesized from pre-β-lipoprotein via an intermediate lipoprotein, which Levy (1971) thinks may well be the 'floating β-lipoprotein' referred to later. Furthermore, Marsh (1971) considers that α-lipoprotein may then be produced in turn from β-lipoprotein since after the administration of heparin (which reduces plasma pre-β-lipoprotein levels) there is a concomitant rise in α- and β-lipoprotein concentrations.

After any meal the serum lipoprotein concentration rises and takes 8–10 h to drop to a steady level. Hence it is essential to obtain serum after a 12–14 h fast (Levy & Fredrickson, 1968) and values quoted without this precaution should be disregarded.

All four lipoproteins are frequently referred to by other names (see Table 1).

TABLE 1

Lipoprotein	Other terminology		
Chylomicrons	Chylomicrons	S	10^4–10^5
Pre-β-lipoproteins	V L D L	S	20–400
β-lipoproteins	L D L	S	0–20
α-lipoproteins	H D L	S	2

TABLE 2

Type	Lipoprotein in excess	Net result in fasting serum
I	Chylomicrons	Normal or high cholesterol High triglycerides
IIa	β-lipoprotein	High cholesterol Normal triglycerides
IIb	β-lipoprotein Pre-β-lipoprotein	High cholesterol High triglycerides
III	'Floating β'	High cholesterol High triglycerides
IV	Pre-β-lipoprotein	Normal or high cholesterol High triglycerides
V	Chylomicrons Pre-β-lipoproteins	Normal or high cholesterol High triglycerides

In 1967 Fredrickson and his colleagues showed that some people with xanthomatosis produce an abnormal lipoprotein which they termed 'floating β' lipoprotein because of its properties in the ultracentrifuge. It is also frequently called 'broad β' lipoprotein because it appears as a broad diffuse band on electrophoresis, and seems to contain a large amount of cholesterol and triglycerides.

The classification of the clinical types of hyperlipoproteinaemia recommended by the World Health Organization (1970) is given in Table 2, and is based on Fredrickson's classification which contained only one Type II. Strisower, Adamson & Strisower (1968) modified the classification to include another type—Group 5. In the opinion of the World Health Organization (1970) and Stone *et al.* (1971) this is really Type II(b), a group which seems to occur within a family already having Type II(a) amongst its members. Both Strisower and Stone are of the opinion that Frederickson's Type V is really a sub-group of Type IV.

Reviews by Stone & Levy (1972) and Slack & Borrie (1971) give the correlations between the clinical findings and the biochemical data in these types of hyperlipoproteinaemia.

It is clear that the classification depends upon the serum lipoprotein concentration since it is obviously possible for a high cholesterol and a high triglyceride concentration to occur together in more than one type. Originally, lipoprotein concentrations were determined by means of the ultracentrifuge but this machine is too expensive for most laboratories. So much attention has been given to the use of zone electrophoresis for the identification and quantitation of the lipoproteins, using paper (Lees & Hatch, 1963), cellulose acetate (Chin & Blankenhorn, 1968) and agarose gel (Noble, 1963). No doubt the recently introduced polyacrylamide gel technique of Frings, Foster & Cohen (1971), will have its adherents. Nevertheless, whilst chemists consider all these methods are reasonable for qualitative work, most treat quantitative electrophoresis with reserve because of the large errors inherent in the technique.

In this country Stone & Thorp (1966) have shown that by using a specially constructed micronephelometer in conjunction with a cholesterol determination, serum lipoprotein concentrations can be measured quantitatively and with ease, and that provided certain precautions are taken, these results correlate well with those obtained by the analytical ultracentrifuge (Thorp, 1969; Stone *et al.*, 1970). Furthermore, the serum triglycerides can be calculated and agree well with those obtained by other methods (Buckley *et al.*, 1970). The disadvantage of the technique is that where the serum is very turbid as in Type III, it needs to be diluted beyond the limit set for the accuracy of the instrument. As a result, the serum from such a type appears to be a Type IV. However, Stone *et al.* (1970) have established the criteria which must be fulfilled if the serum is to be a Type III.

The treatment of cutaneous xanthomata is not easy, for although the serum lipoprotein concentrations can often be brought down to normal or near normal levels by the use of the appropriate diet, the xanthomata do not always disappear. Eruptive xanthomata seem to resolve easily (Fredrickson *et al.* 1967; Levy & Langer, 1969), tuberous and tendinous taking more time to go (Slack & Borrie, 1971). Only one case is recorded where xanthelasma has resolved (Palmer & Blacket, 1972).

It is unfortunate that Types I–IV are hereditary and that the last three types are prone to atherosclerosis. Paterson & Slack (1972) show that there is a high risk of morbidity amongst men with Type II lipoproteinaemia, but although there is a high incidence of coronary heart disease in Types III–V, the death rate is low.

Evidence that the requisite diet with or without drug therapy prevents atherosclerosis is sparse although Zelis *et al.* (1970) claim that in six patients with Type III, institution of dietary therapy in conjunction with clofibrate improved the blood flow in the leg arteries.

Clofibrate, D-thyroxine, cholestyramine and nicotinic acid can all be used to lower the cholesterol concentration. Clofibrate is thought to interfere in the synthesis of cholesterol at the stage of conversion of acetyl-CoA to mevalonate (Sodhi *et al.*, 1971), and it is known to lower the triglyceride level by interfering with the binding of free fatty acids to serum albumin (Bridgman, Rosen & Thorp, 1972). D-thyroxine is thought to increase the catabolism of cholesterol (Krikler, Lefevere & Lewis, 1971). Cholestyramine removes bile acids in the gastrointestinal tract by adsorption, thus diminishing the pool of available cholesterol in the liver (Fallon & Woods, 1968). (It is possible that the recently introduced DEAE Sephadex therapy of Howard & Hyam (1971) will work in a similar fashion.) Nicotinic acid is thought to inhibit cholesterol synthesis (Hardy, Gaylor & Baumann, 1960) and to cause further loss by increasing the excretion of neutral steroids of endogenous origin (Miettinen, 1968). It also inhibits the mobilization of free fatty acid from adipose tissue thus reducing the endogenous synthesis of triglycerides (Carlson & Orö, 1962).

The diet for Type I is aimed at reducing the fat intake, as these patients have an inherent lack of one of the lipoprotein lipases, the enzymes necessary for clearing the chylomicrons from the plasma. A totally restricted fat diet is unpalatable, but Greenberger & Stillman (1969) have an ingenious way of circumventing this by giving triglycerides containing medium chain lengths of eight or ten carbon atoms. By this means not only is the palatability of the diet improved, but these fats can be absorbed directly without chylomicron formation. Furthermore, they facilitate the absorption of the fat soluble vitamins which are necessary for the children in whom this rare type of hyperlipoproteinaemia occurs.

The nature of the underlying disease in Type II(a) or II(b) is obscure. Langer, Strober & Levy (1972) have presented data suggesting that it is caused by an impairment in the catabolism of β-lipoprotein. In any case a low cholesterol diet helps. This is achieved by limiting the amount of dietary fat from mammalian sources. The use of polyunsaturated fats of vegetable origin is recommended. Although these contain less cholesterol than those of animal origin the precise means by which the serum cholesterol is lowered is not understood (Greenberger & Stillman, 1969). Cholestyramine can be used to lower the serum cholesterol and is frequently given in conjunction with a low cholesterol diet.

Despite the fact that clofibrate and D-thyroxine interfere with cholesterol metabolism, neither are particularly effective in cases of Type II, implying that there may be more than one underlying defect in the cholesterol metabolism in such patients.

Hypothyroidism can give rise to a Type II pattern complete with xanthomata, due to a defect in the removal of β-lipoprotein and cholesterol (Walton *et al.*, 1965). Suitable therapy reduces the β-lipoprotein level to normal, concomitantly lowering the serum cholesterol.

Unlike Types I and II, Types III–V are carbohydrate dependent. Excess plasma pre-β-lipoproteins might obviously arise from over-production or under-utilization and there is doubt as to which is the cause (Reaven *et al.*, 1965; Havel *et al.*, 1970). Normally, the triglycerides will be derived from glucose or fatty acids of dietary or tissue origin. The excess fatty acids produced in uncontrolled diabetes mellitus and alcoholism, will also increase pre-β-lipoprotein synthesis and explains the finding that uncontrolled diabetics and alcoholics frequently exhibit xanthomata and have Type IV hyperlipoproteinaemia. There appears to be no explanation of the known association of diabetes mellitus secondary to hyperlipoproteinaemia in Types II, IV and V (Nikkilä, 1969).

Type III is thought to be caused by an inability to catabolize the intermediate lipoprotein formed during the breakdown of pre-β-lipoprotein to β-lipoprotein (Levy, Bilheimer & Eisenberg, 1971).

All three types respond dramatically to a restricted carbohydrate diet, particularly if the patient is obese. Where the patient has glucose intolerance, and/or, a high serum cholesterol, these too are improved. Clofibrate and D-thyroxine are useful adjuncts to the diet, since both will lower the serum cholesterol and the triglyceride levels.

In the rarely occurring Type V, the presence of chylomicrons is thought to be due to a lowered activity of lipoprotein lipase (Greten, Levy & Fredrickson, 1969). Consequently, a diet rich in polyunsaturated fats of medium length carbon chains needs to be given in conjunction with one restricted in carbohydrates.

Finally, it should be pointed out that hyperlipoproteinaemia can occur in conjunction with xanthomatosis in dysglobulinaemia and biliary obstruction. In the case of the dysglobulinaemias, the cause is unknown; in the case of biliary obstruction the cause is thought to be due to the overloading of lipoprotein with lipid giving rise to an abnormal lipoprotein (lipoprotein X), secondary to biliary retention (Rifkind, 1971). When the primary condition is alleviated, the lipoproteinaemia and the xanthomata disappear.

Department of Dermatology
University of Liverpool

F. DOROTHY DEAN

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British Journal of Dermatology (1973) 88, 197.

Obituaries

P. B. MUMFORD

Dr Percival Brooke Mumford, former consultant dermatologist to the United Manchester Hospitals, Manchester and Salford Hospital for Skin Diseases and Macclesfield Infirmary, and lecturer in dermatology at the University of Manchester, died, at the age of 76, on 10 June 1972.

The son of a famous father, Dr A.A.Mumford, who was a well-known general practitioner in Manchester, local historian and Medical Officer at Manchester Grammar School for many years, P.B. Mumford was born on 17 April 1896. After attending Manchester Grammar School he became a medical student at Manchester University. In 1915 he interrupted his studies and served in the first world war as an officer in the Royal Field Artillery on the French and Italian fronts. Returning to the University in 1918 he qualified M.B. Ch.B (Manc.) with honours in 1920. Following a house job at the Royal Infirmary he then began a lifelong association with the Manchester and Salford Hospital for Skin Diseases, first as assistant medical officer for 10 years, then as honorary assistant physician and later as full physician and finally as senior physician. He was also, at varying times, visiting pathologist at Macclesfield Mental Hospital, pathologist to the Veneral Diseases Department at Manchester Royal Infirmary and later physician in the same department. From 1929 to 1938 he was also honorary dermatologist at Warrington and Park Hospital, Davyhulme. He proceeded M.R.C.P. in 1922., M.D. (Manc.) in 1925 and F.R.C.P. in 1932. He became head of the department of dermatology at the Manchester Royal Infirmary in 1931, succeeding the late Dr Dyson, who had been the first holder of the post from 1923. In 1961 he retired from hospital work under the age ruling, but continued in private practice till a few days before his death. He acted as consultant to the Ministry of Pensions from 1924 to 1931, and for many years served on the Medical Tribunal for the Ministry of Social Security until compulsorily retired at the age of 74. For very many years, up to his death, he was medical referee for Manchester Crematorium. He served as honorary secretary of the Manchester Dermatological Society from 1922 till 1929, then as secretary of the newly formed North of England Dermatological Society during 1929 and 1930. In 1939 he was President of the Society and was largely responsible for re-starting the Society after the war in 1947, being elected president again for that year.

Dr Mumford's services to the community and to dermatology in the North of England were unique. His name became a household word among patients, general practitioners, solicitors, trade unions and insurance companies, making him the first choice in clinical and medico-legal work in all aspects of his specialty. As a result his private practice became enormous and his clinics at hospitals so popular that he sometimes dealt with nearly 100 patients in a single morning. Critics might complain that these clinics did harm to the image of dermatology, but the patients did not complain and were able to obtain an expert opinion at short notice when it was most needed. His reputation was built on exceptional diagnostic and therapeutic skill based on a vast knowledge of his subject. He was never ruffled or disconcerted, and so inspired confidence in the patient which contributed largely to the high success rate of his case management.

Perhaps his greatest contribution to dermatology in Manchester was not his pioneer work with thorium X, or his research into more acceptable and efficient ointment bases, but his far-seeing belief that all the consultants in his specialty working in Manchester should be equal members of the staff at the Manchester and Salford Skin Hospital. He fought for this principle long before the National Health Service began, when consultants frequently believed that those in the same specialty were rivals, not colleagues. The result was a harmony and successful relationship continuing to this day, as the principle was accepted by the Regional Hospital Board in 1948 and never changed. Another field in which his far-sightedness bore fruit was his pioneering advocacy of co-operation between radiotherapists and dermatologists in the treatment of skin malignancies. He established such happy relationships with the Christie Hospital in Manchester that a weekly clinic, controlled by a radiotherapist, has been held at the Manchester Skin Hospital for nearly 40 years. In recognition he was elected an honorary fellow of the Faculty of Radiologists.

In his youth he was a keen sportsman, especially enjoying playing cricket and golf, as well as being a useful hurdler on the athletic field. He took a lively interest in boxing and enjoyed many an evening at Belle Vue watching the bouts. As a student he edited the *Manchester Medical Gazette* and played his full part in other student activities.

As a teacher he enjoyed lecturing, and countless students will recall his unlimited supply of stories from personal experience. He could inspire loyalty of a very high order, patients often corresponding with him for years and long after their skin trouble had recovered. His personal staff showed equal loyalty, as did his colleagues.

A devoted family man, he celebrated his golden wedding a few years ago and is survived by a son (medically qualified) and two daughters (one also qualified and the other a physiotherapist.)

GEOFFREY AUCKLAND

R.D.MOYLE

Dr R.D.Moyle, formerly Dermatologist to the Eastbourne Group of Hospitals and the Queen Victoria Hospital, East Grinstead, died on 4 April 1972.

Robert Davies Moyle was born in Brantford, Ontario, in 1886. From McMaster University, Ontario, where he graduated B.A. in 1908, he entered the family business. Two years later he decided that medicine was to be his future career and gained a place at Johns Hopkins University, where he qualified M.D. in 1914. The following year he joined the Canadian Army Medical Corps and was awarded a Military Cross in 1918. In 1916, during a period of leave, he obtained the Conjoint diploma. On demobilization he was appointed Deputy Superintendent of Queen Alexandra's Sanatorium, Ontario. In 1924 he returned to the United Kingdom and entered general practice in Hastings before moving to Eastbourne. It was during this period that he developed his interest in dermatology and gained experience in the specialty with clinical attachments at the London Hospital and the former Skin Hospital in Fitzroy Square.

Though well past military age, Moyle served as a Major in the R.A.M.C. from 1941 to 1946 as a dermatological specialist. After the Second World War he was appointed to the Queen Victoria Hospital, East Grinstead, Bexhill Hospital, and the Eastbourne Group and continued in these appointments until his retirement in 1958. He is survived by his widow and a son who is a Consultant Radiologist.

P. HALL-SMITH

Personal Appreciation

JOHN T. INGRAM

It was with great sorrow that I read again of the passing of another old friend and colleague, Jack Ingram as he was known to his friends, so shortly after reading of the passing of Tom Brain, in the *British Medical Journal*. He was well known in this country as a co-author with Tom Brain, of Sequeira's *Diseases of the Skin* and for many other contributions to dermatological literature. I first met him personally at a meeting of the British Association of Dermatology in Belfast in 1948, following which he invited me to stay with him in Leeds. We became firm friends thereafter and I stayed with him on other occasions and had the pleasure of having him and his wife and daughter Pamela stay at our home in Sydney. During his visit, he made many Australian friends both dermatological and otherwise. He was among the first three British Honorary Members of our Dermatological Association of Australia and became a Corresponding Member of the Australasian College of Dermatologists when it was formed in 1967. He was one of the dermatological 'greats' of his time, and not the least of his accomplishments (in fact, probably the greatest), he founded and occupied the first Chair of Dermatology in England. This was a very fine feat in view of the general lack of appreciation of the importance of dermatology in the practice of medicine.

Jack was a kind and lovable personality, with quite outstanding brilliance in dermatology, as his clinics and writings indicated so well.

He will be much missed by all his Australian colleagues who I know, will join me in expressing our great sorrow at his loss to his family and British colleagues. In fact, medicine as a whole, will be noticeably the poorer for his passing.

JOHN C. BELISARIO

Society Proceedings

Royal Society of Medicine

SECTION OF DERMATOLOGY

President: Dr H. R. Vickers
Thursday 20 April 1972

Eosinophilic facial granuloma

DR P. D. SAMMAN AND DR D. C. G. BETT

A man aged 45.

History. The lesion on the nose was first noticed in 1967. It extended for a time and then became stationary. Biopsy showed a granuloma but at that time the lesion was not thought to be an eosinophilic granuloma. A few small doses of superficial radiotherapy were given. The response was slow but over the next 12 months the condition slowly regressed leaving a slightly depressed scar. In June 1971 there was a relapse and the plaque returned to its previous size. It was symptomless.

On examination. There is a brown plaque on the left side of the nose, slightly infiltrated and with some large blood vessels on the surface.

Investigations. Two further biopsies have been done and Dr E. Wilson Jones now reports on the third as follows: 'The histology is very similar to the two previous biopsies. The most striking feature is the dense granulomatous infiltrate with numerous plasma cells and the formation of lymphoid follicles. In places polymorphs and eosinophils are also seen. The histology is certainly unusual for a straightforward case of eosinophilic granuloma but I have encountered this type of histology in long standing examples of the condition'.

Course. The patient is now being treated with topical steroid applications and the condition is improving.

Comment. The main interest here is the scarcity of eosinophils in a case which in other respects is characteristic.

Yellow nail syndrome treated by intralesional triamcinolone acetonide

DR E. ABELL AND DR P. D. SAMMAN

Case 1. A man aged 62.

History. Yellow, thick, slow growing nails had appeared on all fingers and great toes for 1 year. Occasional shedding of a nail occurred.

On examination. Thick, yellow-green, biconvex nail plates with transverse ridging, variable onycholysis and absence of both lunule and cuticle were seen on all affected digits (Fig. 1). Marked erythema and oedema of the posterior nail fold was also present on all but no oedema of limbs or face could be demonstrated.

Investigations. Hb, WBC, ESR and CXR normal. Mycological examination normal.

Case 2. A man aged 62.

History. For 2 years all finger nails had become thick, yellow and slow growing. Shedding of nails occurred occasionally. Chronic bronchitis for 12 years.

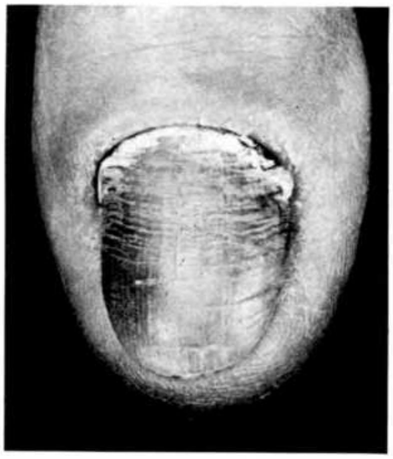

FIGURE 1.

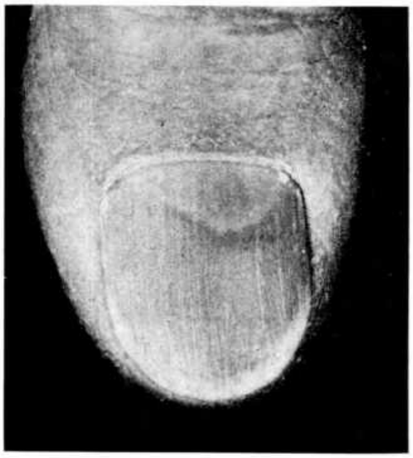

FIGURE 2.

On examination. All fingers showed changes of the yellow nail syndrome with oedema of posterior nail fold and also of the left ankle.

Investigation. CXR normal. Mycological investigation normal. Nail growth rate (average) 20/μm/day.

Treatment. Triamcinolone acetonide at a concentration of 5 mg/ml was injected into the posterior nail folds of all affected nails of both patients using a needleless injector (Porto-Jet) as described by Abell (1972), initially at 2 weekly intervals on three occasions and subsequently every 6–8 weeks. The fingers of the left hand of Case 1 were treated first and the right hand nails only after signs of improvement in the former.

Result. After 6 months all the left hand nails of Case 1 began to grow out normally and treatment to right hand nails was followed by their recovery in a further 4 months. The nails remained normal 10 months after cessation of injections (Fig. 2). The great toe nails remain affected. Both hands of Case 2 were treated and the first sign of normal nail appeared after 6 months in seven nails. By 8 months normal appearing nail was growing from all ten nail folds.

Comment. This investigation was prompted by a personal observation (Samman) of recovery of a patient's yellow nails after systemic steroid treatment of his asthma and bronchitis. Spontaneous recovery in this syndrome is unusual and the recovery of all the nails of these two patients after a similar period of treatment suggests a definite therapeutic effect, albeit indirect. The association of lymphatic abnormality has been described with varying incidence by Samman & White (1964) and Marks & Ellis (1970) but whether this feature is aetiologically of importance in production of the yellow nails is unknown. It is difficult to postulate a reason why triamcinolone would produce a beneficial effect if lymphatic hypoplasia were the primary defect.

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SAMMAN, P.D. & WHITE, W.F. (1964) The 'yellow nail' syndrome. *British Journal of Dermatology*, **76,** 153.

The following cases will be published in full in *Proceedings of the Royal Society of Medicine*

Urticaria pigmentosa with spinal osteoporosis
DR D. R. IVES AND DR D. M. THOMPSON (for PROFESSOR C. E. DENT AND DR T. W. E. ROBINSON)

Late congenital syphilis untreated until Adulthood
DR A. N. MCCLEAN (for DR J. K. OATES)

Practolol-induced drug eruption
DR E. S. K. ASSEM AND DR R. A. BANKS

Chromoblastomycosis
DR WARWICK L. MORISON

Eastern Counties Dermatology Society

4 March 1972, Cambridge
Chairman: Dr R.H.Champion

Hidrocystoma

DR A.MACMILLAN for DR S.O.B.ROBERTS.

A man aged 50.

Clinical features. On the medial part of both lower eyelids he has multiple grouped translucent cysts which release a clear fluid when punctured, present for 4 years (Fig. 1). No aggravating factors.

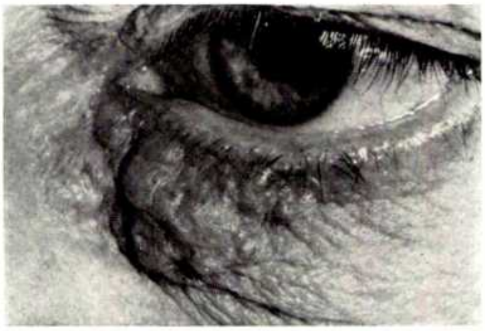

FIGURE 1.

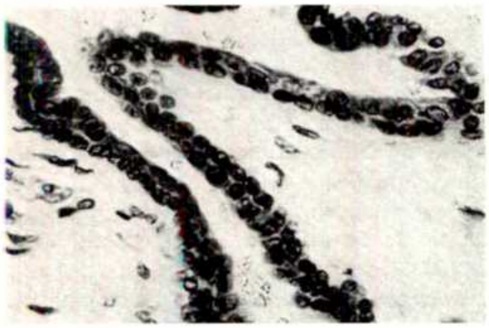

FIGURE 2.

Histology. There are anastomosing cystic spaces in the dermis which is oedematous and contains extravasated erythrocytes. The cyst wall is a double layer of flat to columnar cells resembling eccrine ducts or tubules (Fig. 2).

Comment. Hidrocystoma is a rare, clinically diagnosable sweat gland tumour. This case is atypical in that the patient was male, he did not work in a hot environment and that the lesions were not aggravated by heat.

REFERENCE

DOSTROVSKY, A. & SAGHER, F. (1942) Experimentally induced disappearance and reappearance of lesions of hidrocystoma. *Journal of Investigative Dermatology*, 5, 167.

Still's disease: Rash in mother and daughter

DR B.R.JONES for DR R.H.CHAMPION

Mother aged 26, daughter aged 10 months.

Clinical features. Mother—since the age of 2 she has had a profuse macular erythematous rash which recurs every day at different sites. The individual lesions are irregularly shaped, up to 2 cm across and last up to 24 h. A few are slightly raised, not urticarial and moderately itchy. From 2 to 10 years of age she had articular and non-articular 'rheumatism'. She has no joint deformity and now has only occasional pains in her knees and ankles. She has had episcleritis intermittently for many years. She has always been anaemic. Daughter—rash similar to mother's from birth: otherwise well.

Investigations. (Mother repeatedly over 22 years.) Hb 7·5–10·4 g%, WBC 12,000–20,000, persistent polymorph leucocytosis, ESR 80–100 mm/h, serum iron 65 μg/100 ml, serum proteins—raised alpha 2, beta and gamma globulins, Rose Waaler, LE cells, ANF—negative, X-rays of joints and chest—normal.

Comment. There is an increased familial incidence of Still's disease (Ansell, Bywaters & Lawrence, 1969). The rash of Still's disease (Isdale & Bywater, 1956; Bywaters, 1971) in parent and child without joint involvement and the onset from birth has probably not been reported before.

REFERENCES

ANSELL, B.M., BYWATERS, E.G.L. & LAWRENCE J.S. (1969) Familial aggregation and twin studies in Still's disease. *Rheumatology*, 2, 37.

BYWATERS, E.G.L. (1971) Still's disease in the adult. *Annals of Rheumatic Diseases*, 30, 121.

ISDALE, I.C. & BYWATERS, E.G.L. (1956) The rash of rheumatoid arthritis and Still's disease. *Quarterly Journal of Medicine*, 25, 377.

Livedo with ulceration

DR B.R.JONES for DR A.J.ROOK

A woman aged 38.

Clinical features. An ulcer developed following an acute paronychia of the right big toe nail in September, 1970 and subsequent removal of the nail. From February to June 1971 she spent two periods in another hospital where a biopsy showed chronic inflammatory changes. She was treated with prednisone with some temporary benefit. When first seen in October, 1971 there was a sharply defined, indurated ulcer at the base of her right hallux (Fig. 1). She was admitted to hospital where livedo reticularis developed over the lower part of the right leg (Fig. 2). The original ulcer healed but three small necrotic areas appeared lateral to its site in February 1972. These have now coalesced. General eximination has been normal. Blood pressure 125/80.

Investigations. The following gave normal results: Hb, WBC, ESR, MSU, serum electrolytes, urea, plasma proteins + electrophoretic strip, liver function tests, WR, X-ray of chest and right foot, arteriogram.

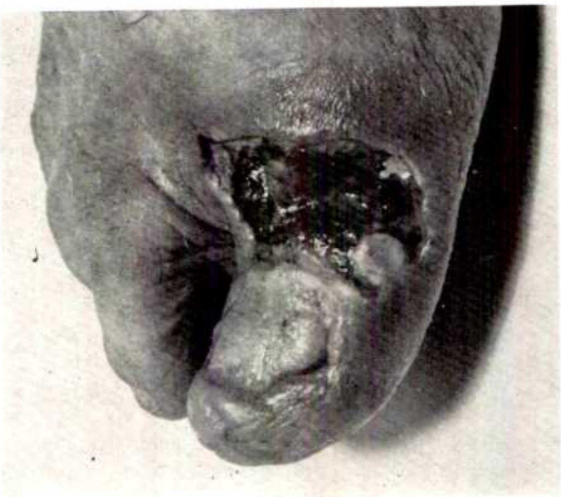

FIGURE 1.

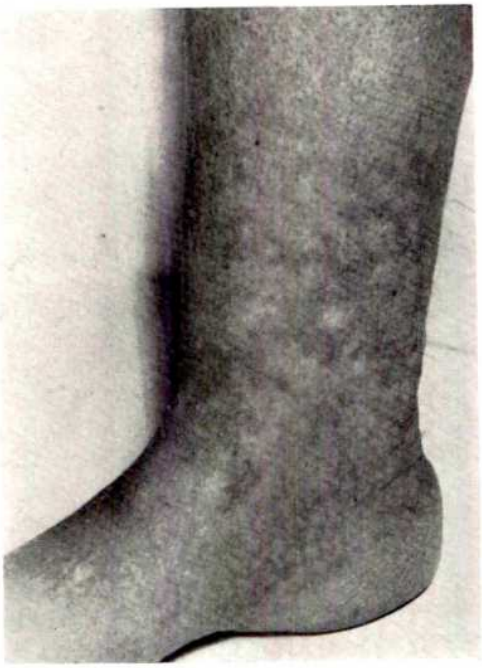

FIGURE 2.

Culture of skin biopsy—*M. tuberculosis* not isolated, no fungi grown. No evidence of haemoglobinopathy. Histology of ulcer—the deeper layers contain many lymphocytes, occasional histiocytes and a few giant cells arranged around blood vessels.

Comment. The appearance of the ulcer strongly suggested that it was an artefact and since investigations suggested no other cause this possibility was not ignored until the failure of occlusive dressings to promote healing and the development of livedo reticularis suggested the correct diagnosis. Livedo with ulceration has been successfully treated with azathioprine (Braun-Falco & Meigel, 1972) and this treatment will be considered if the present satisfactory progress is not maintained.

REFERENCES

BRAUN-FALCO, O. & MEIGEL, W. (1972) Zur Azathioprin-Therapie der idiopathischen Livedo racemosa mit Ulcerationen. *Hautarzt*, 23, 136.

Correspondence

CHRONIC MUCOCUTANEOUS CANDIDIASIS AND ALOPECIA AREATA

SIR, In a recent article 'Chronic mucocutaneous candidiasis, endocrine deficiency and alopecia areata' *British Journal of Dermatology* (1972) 86, 238, a family with 'endocrine candidosis syndrome' was described, in which one of the affected members had alopecia areata. Review of the literature revealed alopecia areata in thirteen of forty-two patients with this syndrome. Since publication, two other affected members of this family have developed alopecia areata. Two have eyebrow involvement. It is significant that in this family alopecia areata is restricted to three members who suffer from idiopathic hypoparathyroidism.

Department of Dermatology
University Medical Buildings
Foresterhill
Aberdeen AB9 2ZD

L. STANKLER
P. D. BEWSHER

SHAKESPEARE ON THE SKIN

SIR, I refer to the fascinating paper 'Shakespeare on the skin' by J.A. Cotterill, in the May 1972 issue.

Dr Cotterill writes that 'the many references in the works of Shakespeare to the skin, both normal and abnormal, have received little attention from commentators. The only analysis, albeit oblique, was by Davis (1969). . . .'

I feel I should draw attention to the paper 'Shakespeare—the dermatologist' by A.H. McGeoch, published in the *Australian Journal of Dermatology* (1955) 3, 53. I have done this in fairness to the author, who is probably too modest to do so himself.

143 Macquarie Street
Sydney 2000

ADRIAN JOHNSON
Editor, Australian Journal of Dermatology

THE EVOLUTION OF CUTIS MARMORATA INTO NAEVUS ANAEMICUS

SIR, I wish to record the development of a child diagnosed in the neonatal period as having cutis marmorata.

Emma W., was born on 4 April 1969, and was said to be 'blotchy all over' in the neonatal period. When seen by me at the age of 2 months she showed normal skin except for pronounced blotching, with prominent veins, on the left side of the face, and on the left lumbar area. A small telangiectatic naevus was present on the abnormal lumbar area. Subsequent review showed decrease in the clarity of patterning, until in June 1972 at the age of 3 years, the changes in the affected areas were typical of naevus anaemicus. The telangiectatic naevus was unchanged, and the child was otherwise well.

Thus, by clinical diagnosis, an area of localized cutis marmorata has faded, and has left naevus anaemicus.

I have not been able to trace a report of this sequence, although the lesions of cutis marmorata are said to fade by Petrozzi *et al.* (1970); and Moyer (1966) describes the fading of a lesion which did not tan well and which was reported by the mother to 'look transparent' at the age of 3 years. It would seem that the coincidental presence of two rare lesions can be ruled out, and follow up of other patients with cutis marmorata should be made.

Queen Victoria Hospital
Melbourne

DAVID S. NURSE

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MOYER, D.G. (1966) Cutis marmorata telangiectatica congenita. *Archives of Dermatology*, **93**, 583.

PETROZZI, J.W., RAHN, E.K., MOFENSON, H. & GREENSHER, J. (1970) Cutis marmorata telangiectatica congenita. *Archives of Dermatology*, **101**, 74.

Book Reviews

The Nails in Disease. PETER D. SAMMAN (1972) 2nd edition. London: Heinemann. Pp. x+177, 157 illustrations, 4 colour plates. Price £2.50.

The first edition of this book was deservedly popular. The success of the German translation confirmed that the requirements of a wide readership were being met. Yet the few shortcomings assumed an importance proportional to the diversity of disciplines of those to whom the subject was of interest.

In this second edition due cognizance has been taken of these. Slightly rearranged, the text has been revised, brought fully up to date and now includes conditions of importance and interest omitted from the first edition. The simple, clear and endearing style is retained, and a careful selection of type has facilitated quick reference. The overall standard of illustration is now excellent in consequence of inserting many new and deleting a few less satisfactory old figures. There are many more references, an adequate and up to date set concluding each chapter.

In every way this edition is an improvement on, yet is essentially cheaper than, its predecessor. Although no library will be without its copy, this is a book for the individual practitioner to purchase, use and value. He will find it indispensable as a reliable guide and reference book concerned with diseases of or affecting nails.

J. N. S. MITCHELL

Manual of Dermatologic Syndromes. THOMAS BUTTERWORTH (1972) 2nd edition. Philadelphia: Lippincott (distributed in Britain by Blackwell Scientific Publications, Oxford). Pp. 94. Price £1.50.

This small volume enumerates and tabulates the principal clinical features of a very large number of syndromes of which cutaneous lesions are a manifestation. The book fits conveniently into a brief case or even a pocket and may prove useful to the clinician when a particular association of symptoms and signs stirs the sluggish memory but fails to elicit an effective response. However, the application of an acceptable diagnostic label is not an end in itself and the value of this book would have been vastly enhanced without any significant addition to its length or cost if the author had provided each entry with two references to the literature, one to the first description of the syndrome and one to the latest comprehensive review article. Some of the syndromes listed are indeed exotic, and some might defeat even a Parkes-Weber. This wide range should ensure the success of the book in its present form. I hope that the author is already at work on his third edition—with references.

ARTHUR ROOK

Scar Tissue. Its Use and Abuse. J. J. LONGACRE (1972) Springfield, Illinois: Charles C. Thomas. Pp. 170, 88 illustrations. Price £7.75.

This concise and profusely illustrated book represents the culmination of 30 years' work in the field of scar correction. The author is a plastic surgeon and the book is written primarily for surgeons. However, dermatologists could read with advantage those chapters dealing with the formation of hypertrophic scars since they have a particular duty to produce good cosmetic results when carrying out investigative biopsies. Probably none of us can look back over our own records with a perfectly clear conscience. In relating hypertrophic scar formation to malalignment of new collagen fibres in the line of muscle tensile forces, the author is supported by the well-presented studies of Dr Helen Berry and Dr Holmstrand on the metabolism and ultrastructure of collagen respectively. These will be of particular interest to those engaged in the study of collagen. The correction of hypertrophic scars by Z-plasty is, of course, a well-recognized procedure but the support given to it by Dr Berry's studies of the accompanying changes in peptide-bound hydroxyproline and proline excretion provides substantial evidence for the author's thesis.

D. S. WILKINSON

British Journal of Dermatology (1973) **88**, 207.

Quarterly Review

THERAPEUTICS XXI

Deep Venous Thrombosis. Part II—Management

The very high incidence of venous thrombosis in both medical and surgical patients is now established. Recently there has been considerable interest in reducing those factors which predispose to thrombus formation, including venous stasis, vascular damage and blood coagulability. More accurate assessment of the effects of any prophylactic measures is now possible with the diagnostic measures described in our previous article (Edwards, Walker & Frenk, 1972).

PROPHYLAXIS

Physical methods

Leg vein thrombosis quickly occurs in conditions of increased venous stasis, particularly if some veins are occluded by external pressure. These conditions are found during operation and on confinement to bed.

Venous return under these circumstances may be increased by elevation of the legs, by elastic bandaging and by frequent use of the 'muscle-venous' pumping mechanism. Supervision by a physiotherapist is important both for encouragement and to teach correct ankle and leg movements. However, even with a rigorous regime these measures alone have not proved successful in preventing venous thrombosis (Flanc, Kakkar & Clarke, 1969).

Electrical stimulation of the calf muscle intermittently during operation has been tried in two centres (Browse & Negus, 1970; Doran, White & Drury, 1970). In the former trial evaluation was by the ^{125}I fibrinogen technique rather than by clinical assessment. In this series twenty-three patients developed thrombosis in the unstimulated leg whilst nine had thrombosis on the stimulated side. Only two patients developed thrombosis in the stimulated leg alone.

Venous return may also be increased by intermittent external compression of the legs using pneumatic leggings (Hills *et al.*, 1972). Reduction in the incidence of venous thrombosis as measured by ^{125}I fibrinogen uptake was between 17 and 35% in patients without malignant disease, in two different hospitals.

Neither of these methods can be applied to all patients, particularly those with injuries or operations affecting the legs. The compression method has the advantage of being comfortable in use so that it may be used in conscious patients.

Drug prophylaxis

Oral anticoagulants. Oral warfarin failed to reduce the incidence of deep venous thrombosis in patients undergoing orthopaedic surgery (Pinto, 1970) and following myocardial infarction (Murray *et al.*, 1970). In the former instance the drug was given with the premedication.

Heparin. Interest in the use of heparin prophylactically began when Sharnoff, Kass & Mistica (1962) showed that subcutaneous low dosage heparin given for 2–3 days prior to operation was effective in reducing pulmonary embolism.

Kakkar has demonstrated a similar reduction in the incidence of postoperative deep venous thrombosis as diagnosed by the ^{125}I fibrinogen method (Kakkar, Renny & Nicolaides, 1970).

More recently two dosage regimes of heparin have been compared. One group received only three 12 hourly doses of 5000 IU heparin whilst a second group was given 5000 IU 12 hourly for 5 days. The incidence of thrombosis was reported as 42% in the control group and 13·5 and 8·3% in the two test groups respectively (Gordon-Smith *et al.*, 1972).

A large scale trial involving 251 patients undergoing major surgery demonstrated a reduction in venous thrombosis from 24 to only 0·8% in patients given 5000 IU heparin 2 h prior to operation and then 12 hourly for 7 days. Evaluation was again by ^{125}I fibrinogen uptake tests (Nicolaides *et al.*, 1972).

Heparin given in these small doses does not affect the clotting time (Nicolaides *et al.*, 1972) nor does it seem to be associated with any increased risk of haemorrhage.

The mechanism of action of low dosage heparin is not fully understood, but heparin will activate antithrombin III (Yin, Wessler & Stoll, 1971) and the latter is a known inhibitor of factor X (Yin & Wessler, 1970), which is important both in the intrinsic and extrinsic clotting mechanisms. It has also been shown that heparin will alter adenosine induced platelet aggregation (O'Brien *et al.*, 1972).

Factor X is rapidly activated after tissue damage and heparin has failed to reduce the incidence of deep venous thrombosis in patients following myocardial infarction and fracture of the neck of the femur (Kakkar *et al.*, 1972). There is no known contra-indication to low dosage heparin.

Dextran. This substance is a polymer of dextrose molecules which may be combined to form compounds of differing molecular weights. The antithrombotic activity of dextran, molecular weight 70,000 (Dextran 70), was first shown by Borgström, Gelin & Zederfeldt in 1959. Dextran alters the blood clotting mechanism in many different ways. Firstly, it is a plasma expander and this may result in dilution of clotting factors and increased venous blood flow. Secondly, dextran reduces platelet adhesiveness and aggregation in both normal subjects (Bennet *et al.*, 1966) and in postoperative patients (Dhall, Bennet & Matheson, 1968). Platelet interaction with damaged endothelium is also reduced as shown in animal experiments (Berman, 1964; Arfors *et al.*, 1968). Finally, dextran in small amounts will decrease the thrombin clotting time (Laurell, 1951), but the fibrin formed in the presence of dextran is different in texture (Muzaffar *et al.*, 1973).

These changes in fibrin kinetics and structure need further evaluation, but it is possible that such fibrin is less stable and more easily lysed than usual.

Several clinical trials have been performed showing the prophylactic effect of dextran infusions in reducing deep vein thrombosis. Controlled trials (Stadil, 1970; London & Crosfill, 1969; Jansen, 1970 based on clinical assessment alone show a reduction in venous thrombosis from 57 to 20%, 38 to 12% and 9 to 3% respectively. Stadil reported a reduction in pulmonary embolism from 7·3 to 3·1%.

Studies using ^{125}I fibrinogen testing (Huttunen *et al.*, 1971; Bonnar & Walsh, 1972) show similar reductions in deep venous thrombosis. Lambie *et al.* (1970) used patients treated with warfarin given 36 h postoperatively as controls and was able to demonstrate superior prophylaxis with Dextran 70.

Dextran 70 is usually administered by intravenous infusion just prior to, and during operation, being given over 6 h. Further 500 ml units may be given daily for the first 3 postoperative days.

Bleeding has not been a problem with the use of Dextran 70, but the plasma expansion may result in cardiac failure in patients with heart disease.

Aspirin. A trial is at present being conducted by the MRC unit on the prophylactic effect of aspirin in preventing venous thrombosis. The most recent report (*Lancet*, 1972) suggests that the drug is ineffective.

THERAPY OF ESTABLISHED VENOUS THROMBOSIS

Treatment should be aimed at preventing embolization and permanent damage to the vein and its valves. Ideally, therapy should commence as soon as the presence of thrombus is established by phlebography.

Anticoagulants

Heparin given in conventional doses seems to prevent pulmonary embolism and is probably more effective than oral anticoagulants. Dextran may not reduce the incidence of pulmonary embolism (Brisman, Parks & Haller, 1970) though present evidence is contradictory (Stadil, 1970).

None of these drugs affect a thrombus once formed and removal therefore depends upon the body's own lytic mechanisms.

Surgery

The primary aim of surgical intervention is to prevent pulmonary embolism. When thrombus is only present peripherally (below mid-thigh) then femoral ligation is an effective method of controlling repeated small pulmonary emboli; though there is a liklihood of post-phlebitic venous damage. Fresh clot may be expressed from the calf veins through a phlebotomy, which may help to preserve venous valves, but rarely is all the thrombus removed. Recurrent thrombosis is common unless heparin or local fibrinolysis used postoperatively (Mavor & Galloway, 1969).

Ileofemoral and vena caval thrombus can be removed by phlebotomy; though there is a risk of pulmonary embolism unless the vein above the clot is occluded temporarily or permanently (Miles, Chappell & Renner, 1964).

Vena caval ligation does not always control embolism and usually results in serious morbidity in the legs (Mavor & Galloway, 1969).

Thrombolytic therapy

These drugs cause activation of plasminogen to plasmin, which lyses fibrin clot. Urokinase, which is isolated from human urine, is too expensive for induction of generalized fibrinolysis routinely; though it acts quickly and is not antigenic. Streptokinase extracted from streptococcal toxin, activates plasminogen to plasmin indirectly, only working when it is combined to a plasminogen molecule. This complex will then activate eight other molecules of plasminogen to plasmin. For this reason larger doses of streptokinase may be ineffective, since under these circumstances most of the plasminogen is bound to streptokinase and unavailable for activation. Streptokinase causes better resolution of thrombus than heparin, both short term and long term. Venous valves do seem to be preserved by the early use of the drug (Kakkar *et al.*, 1969a, 1969b).

Significant bleeding may occur on treatment with streptokinase. Allergic reactions from a mild pyrexia to anaphylaxis have been reported (Kakkar *et al.*, 1969a, 1969b) and a recent streptococcal infection may result in resistance to the drug so that large initial doses may have to be used.

A commonly used regime is an initial dose of 250,000 units then 100,000 units hourly for 3–7 days, however, we feel that laboratory control is essential because of the complications.

Ancrod

This drug, a purified fraction of Malayan Pit Viper venom, has been found ineffective, compared with heparin in two trials (Kakkar *et al.*, 1969a; Davies *et al.*, 1972).

DISCUSSION

Since modern investigations have confirmed the true incidence of venous thrombosis, it is reassuring to have available effective, safe prophylactic therapy.

Physical methods alone are probably the least effective and least practicable. The excellent results obtained with low dose subcutaneous heparin and Dextran 70, however, demand the use of one of these regimes in all patients considered as high risk with regard to deep vein thrombosis, and possibly in all those undergoing surgery and protracted immobilization.

Patients with either fractured femoral neck or myocardial infarction will not be protected by heparin but Dextran 70 can be given in the absence of heart failure.

Once a thombosis has occurred the risk of pulmonary embolism and post-phlebitic syndrome are the main considerations. Thrombus in the ileofemoral segment should always be treated by fibrinolytic drugs or surgery because of those risks. More peripheral thrombus does not often cause massive pulmonary embolism, though multiple small emboli may result in chronic pulmonary hypertension. Gravitational ulceration, however, is not an uncommon result of peripheral deep venous thrombosis and, whilst it is not possible to predict which patients will develop this complication, it seems reasonable to assume that those with more extensive thrombosis should be offered fibrinolytic therapy.

I. R. EDWARDS

University Department of Therapeutics
Sheffield

ANNE E. WALKER

Royal Hospital, Chesterfield
and the United Sheffield Hospitals

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News and Notices

Royal College of Physicians of London

Members of the British Association of Dermatology elected to the Fellowship in 1972 include John H.S.Pettit.

University of Cambridge Postgraduate Medical School

The fourth of the Courses held at 5 yearly intervals and entitled 'Progress in the Biological Sciences in Relation to Dermatology' will take place in Cambridge from 24 to 29 September 1973. The Course is intended primarily for clinicians of considerable experience, but anyone who is interested is welcome to attend. Details of the programme and accommodation may be obtained from the Secretary of the Medical School, J.W.Woodcock, M.A., Hills Road, Cambridge.

The British Association of Dermatology

The Summer Meeting of the British Association of Dermatology will take place in Cardiff on the 12, 13 and 14 of July 1973 under the Presidency of Dr Geoffrey Hodgson. Papers are invited, and a summary should be submitted of approximately 200 words, precise and factual, avoiding phrases like 'the results will be discussed'. If there is more than one author, please state who is to read the paper. The summary should be in triplicate and should be sent to Dr J.S.Pegum, The London Hospital, Whitechapel Road, London E1 1BB.

International Health Foundation Award—1972

The 1972 Award of the International Health Foundation will go to Dr Herman Musaph of Amsterdam for his paper: 'The psychology of the patient with a skin disease.' Dr Musaph, a psychiatrist, works in the Department of Dermatology at the University of Amsterdam, Binnengasthuis, The Netherlands.

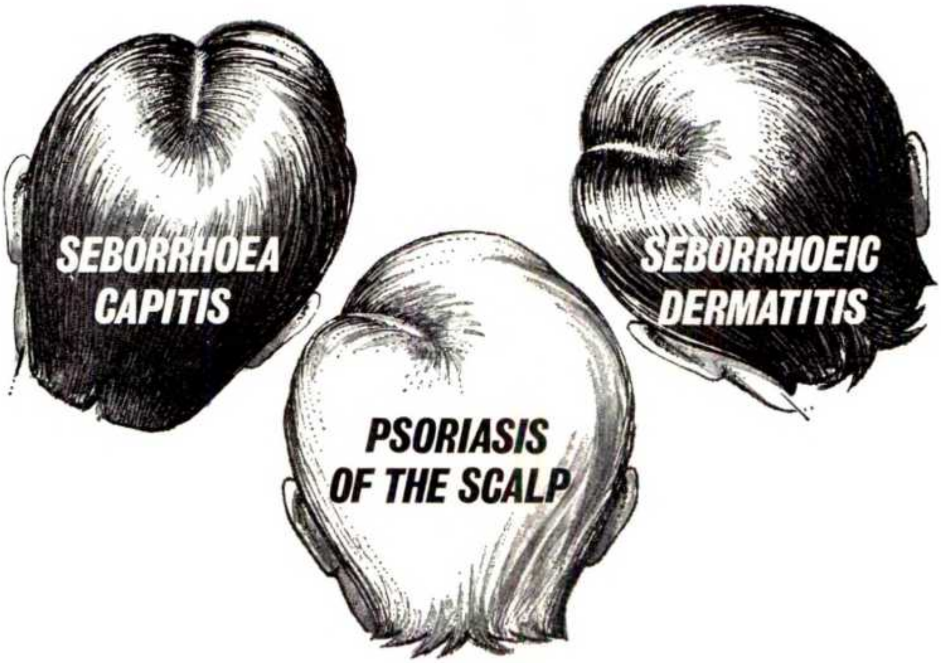
Scottish Dermatological Society

A Meeting of the Scottish Dermatological Society was held in the Western Infirmary, Glasgow, on 12 October 1972. In addition to the clinical section of the Meeting, a guest lecture was delivered by Professor Cormane of Amsterdam entitled 'The challenge of lupus erythematosus'.

Italian Society of Dermatology and Syphilogy

The 50th Meeting of the Society will be held in Naples—Castellammare di Stabia from 10 to 12 May 1973. The main themes will be: (1) cutaneous reactions to drugs; (2) rare photodermatoses; (3) round table on immunofluorescent methods in dermatology.

Enquiries may be addressed to Professor P. Nazzaro, Via San Gallicano 25/a, 00153 Rome.



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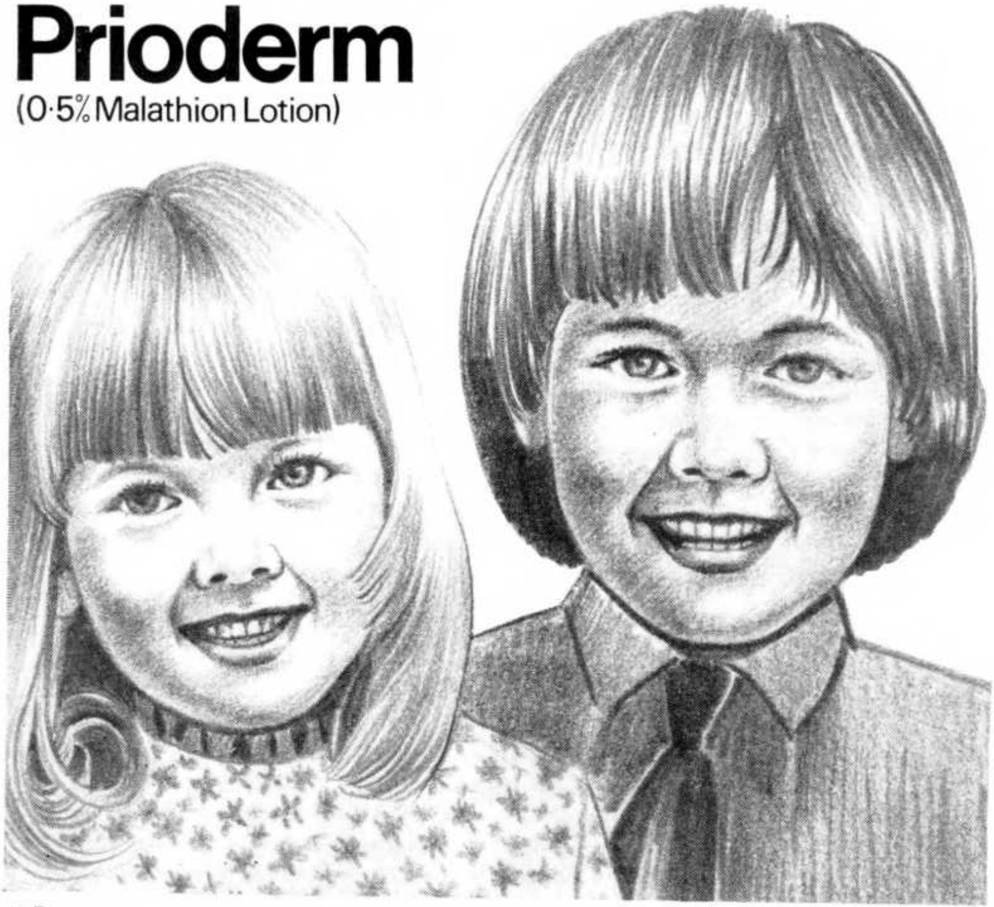

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BOOKS OF SPECIAL INTEREST TO DERMATOLOGISTS

LEPROSY: **Diagnosis and Management (2nd Ed.)** *by* **Harry L. Arnold,** *Jr., Univ. of Hawaii School of Medicine, Honolulu, and* **Paul Fasal,** *Univ. of California Medical School, San Francisco.* Provides basics of etiology, epidemiology, classification, diagnosis and treatment. Common misconceptions of infectivity and transmissibility are dispelled. Special emphasis is placed on diagnostic criteria, selection of biopsy site and biopsy technique. *'73, about 112 pp., 36 il., 3 tables*

KERATINS: **Their Composition, Structure and Biosynthesis** *by* **R. D. B. Fraser** *and* **T. P. MacRae,** *both of Commonwealth Scientific and Industrial Research Organization, Melbourne, Australia, and* **G. E. Rogers,** *Univ. of Adelaide, Australia.* A thorough and up-to-date account . . . collecting together and critically assessing recent relevant results . . . providing a balanced and integrated account of current knowledge of keratins. *'72, 320 pp., 144 il., 24 tables, $16.75*

SOAP PHOTODERMATITIS: **Photosensitivity to Halogenated Salicylanilides** *by* **Peter Simon Herman,** *Univ. of Sherbrooke, Quebec, Canada, and* **W. Mitchell Sams,** Jr., *Univ. of Minnesota, Rochester.* A detailed description of historical, chemical and clinical aspects are given of this entity. It encompasses much of the photobiology phenomena including effects of ultraviolet photons on the skin and chemical compounds. *'72, 192 pp. 24 il. (3 in full color), 2 tables, $14.75*

RADIUM RECIPES FOR CUTANEOUS CANCER **(The Manchester Method)** *by* **J. B. Howell,** *The Univ. of Texas Southwestern Medical School, Dallas. In Collaboration with* **W. J. Meredith** *and* **Gerald Swindell.** Written as a guide for proper application of this method. Reviews the physical aspects of radium and its application to treatment of cutaneous cancer. Recommendations for safe handling, storage of radium, and protection of personnel are emphasized. *'72, 160 pp., 49 il., 2 tables, $14.75*

SCAR TISSUE—ITS USE AND ABUSE: **The Surgical Correction of Deformation Due to Hypertrophic Scar and the Prevention of Its Formation** *by* **J. J. Longacre,** *Univ. of Cincinnati College of Medicine. Contribution by* **Helen K. Berry.** Covers: Embryology of the skin, histology, wounds, pathogenesis of hypertrophic scar, burn scarring and its resulting deformation, correction of contractures, collagen metabolism, etc. *'72, 192 pp. (7 × 10), 449 il., 8 tables, $18.50*

SEXUALLY TRANSMITTED DISEASES *compiled and edited by* **Leslie Nicholas,** *Hahnemann Medical College and Hospital, Philadelphia. Foreword by* **Herman Beerman.** *(22 Contributors)* An up-to-date survey which includes expanded views on sexual diseases which until recently were considered non-venereal. Presentations are given on the sociological, immunological, diagnostic and therapeutic aspects of syphilis and the treponematoses. Viral diseases, and tests are presented. *'73, about 232 pp., 42 il., 29 tables*

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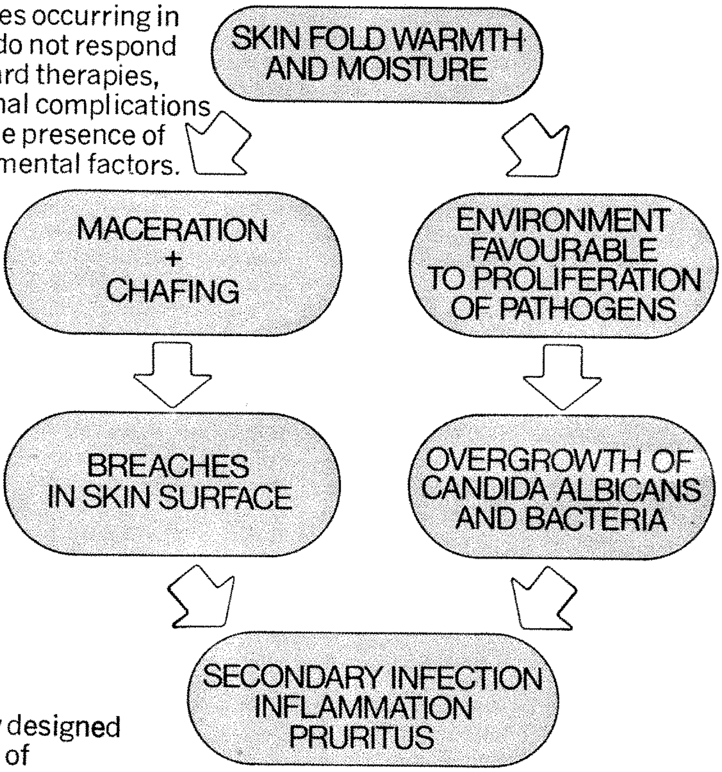
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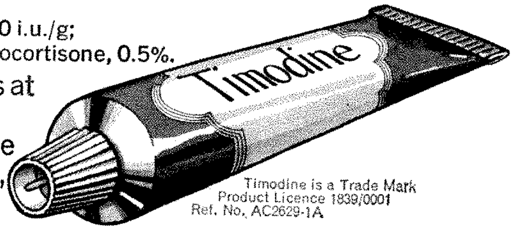

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also quarterly reviews of recent advances in contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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Manuscripts should be sent to the Editor, Dr A. J. Rook, Old Hall, Steeple Bumpstead, Haverhill, Suffolk. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

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kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	µg	cubic millimetre(s)	mm^3	micrometer(s)	µm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	percent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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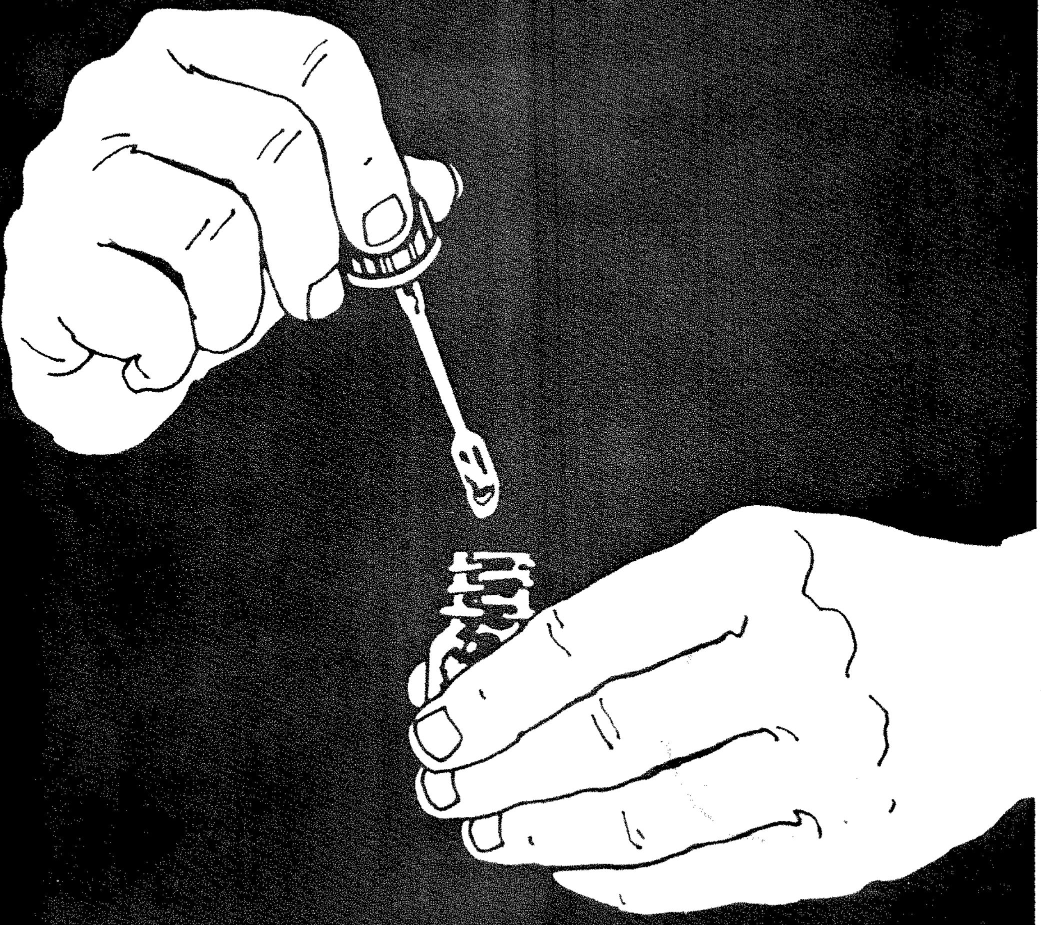

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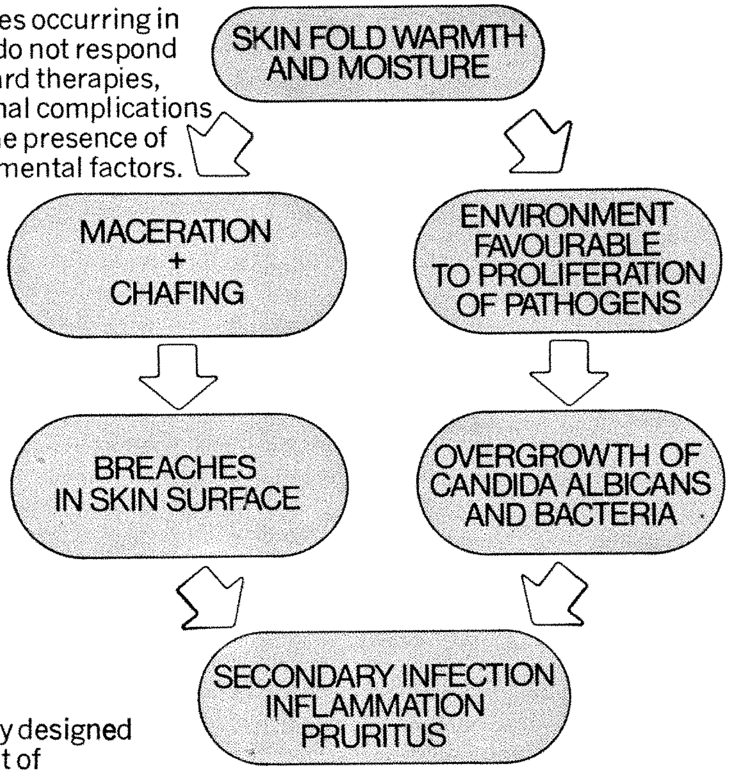
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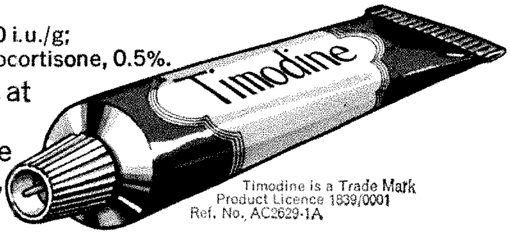

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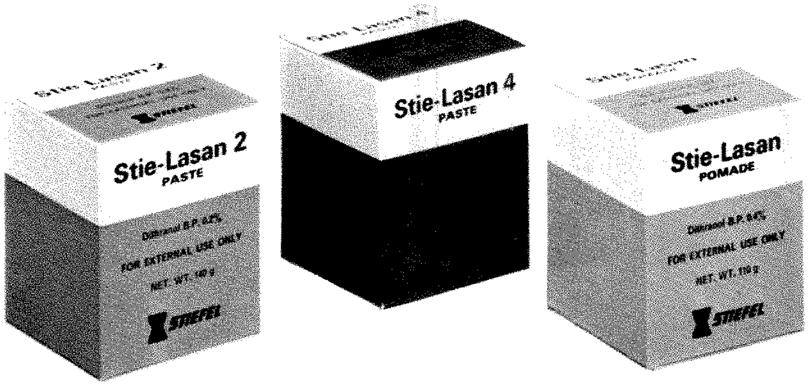

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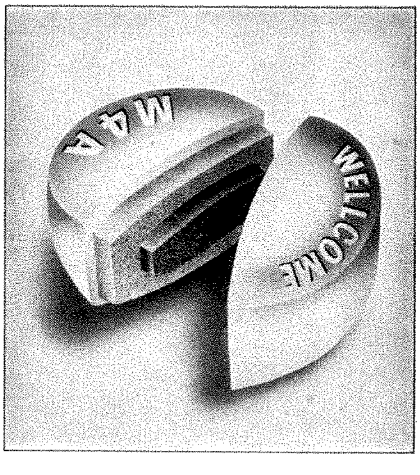
†*Eur J clin Pharmacol* (1971), **3**, 215.



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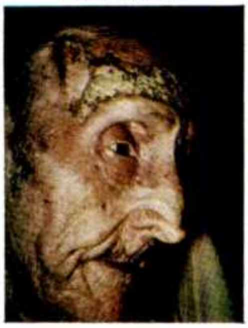

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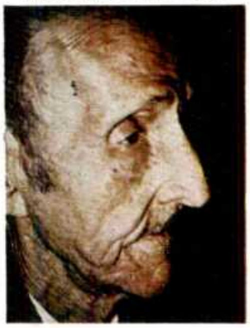

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1. Millikan L.E. and Anderson P.C., Miss.Med., 1971, *68*, 33.
2. Litwin M.S., J.La.med.Soc., 1972, *124*, 33.

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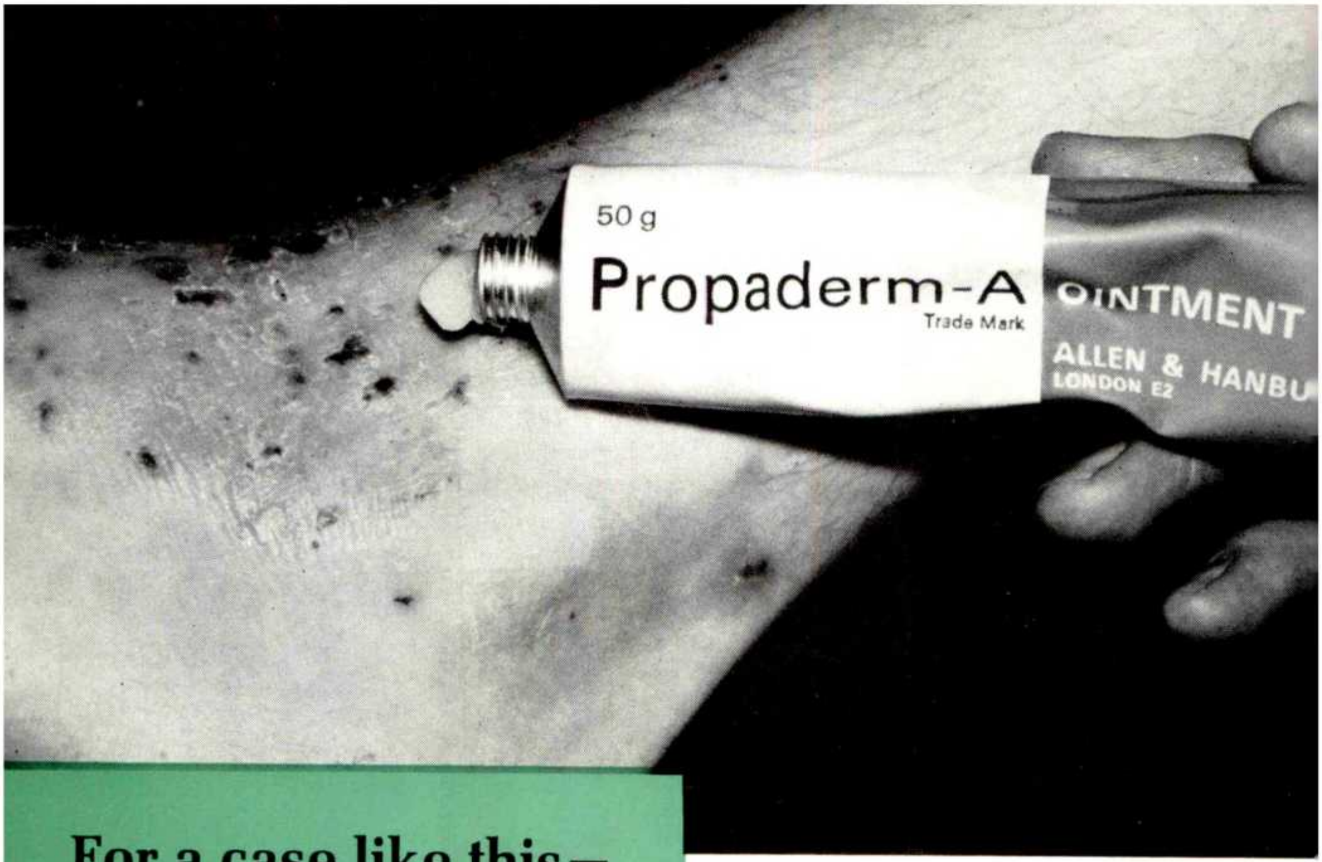

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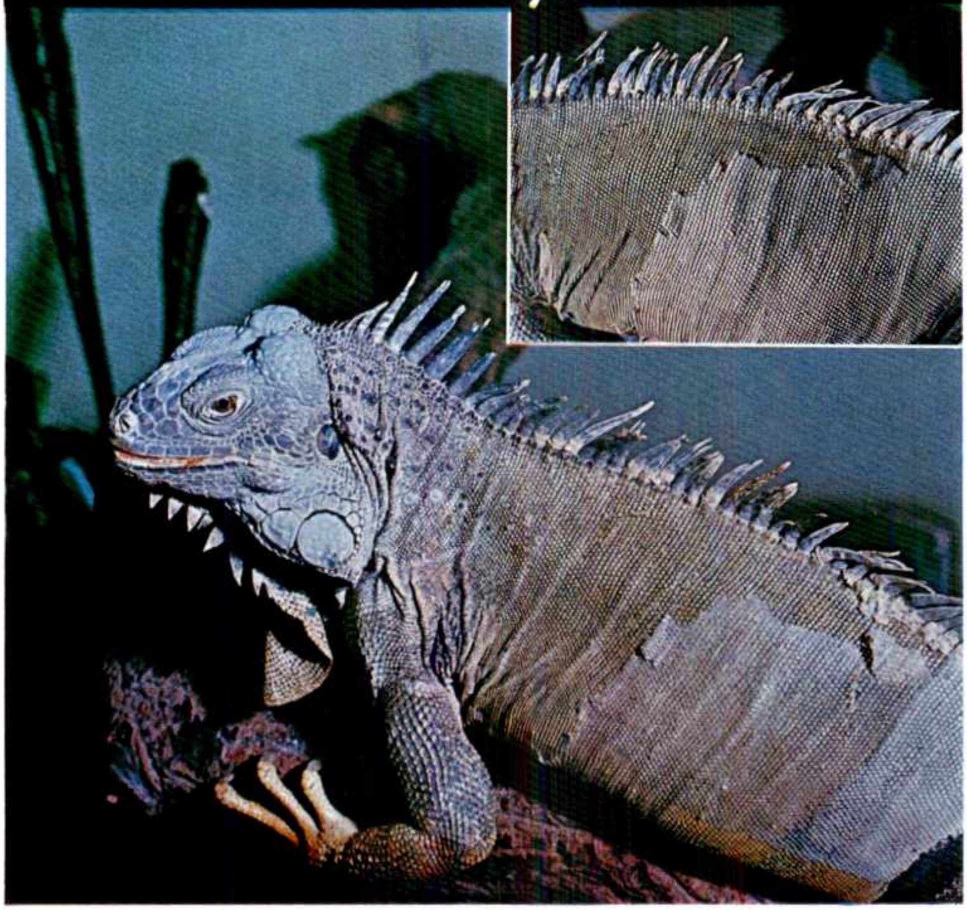

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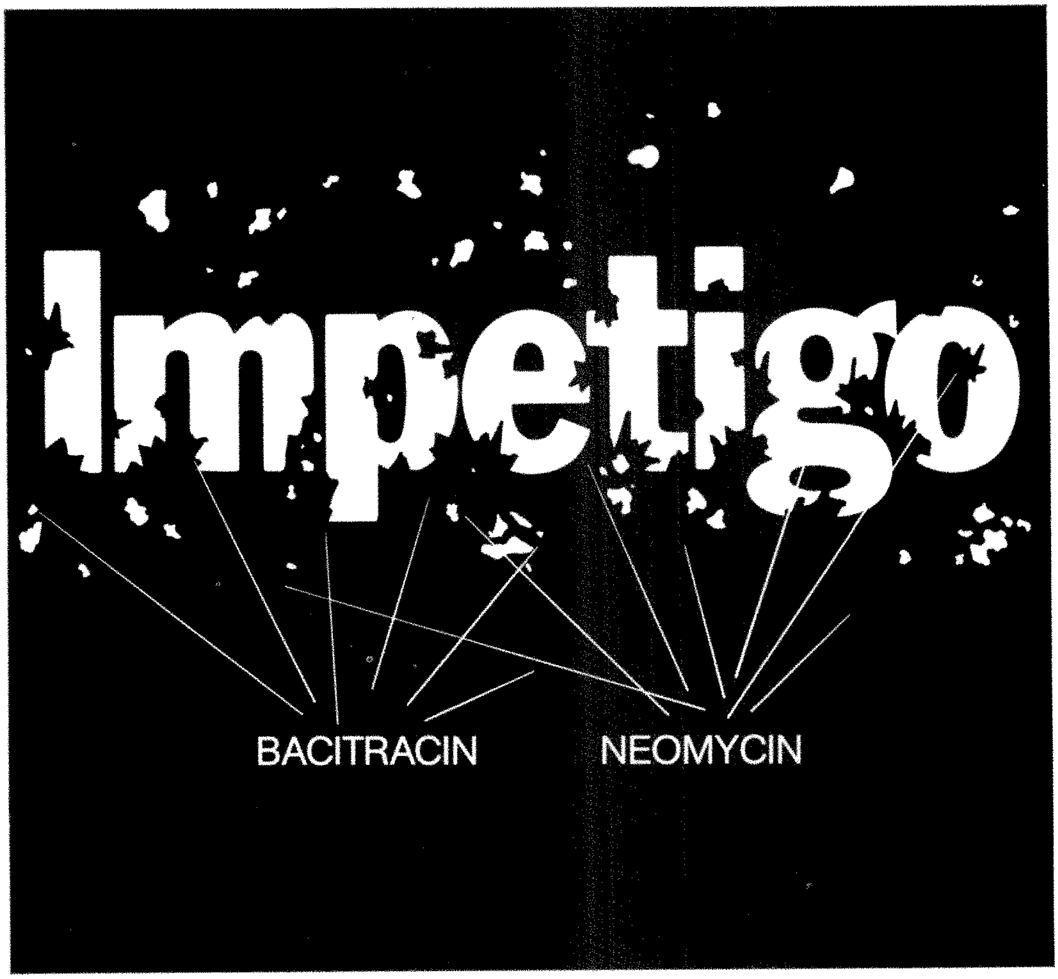

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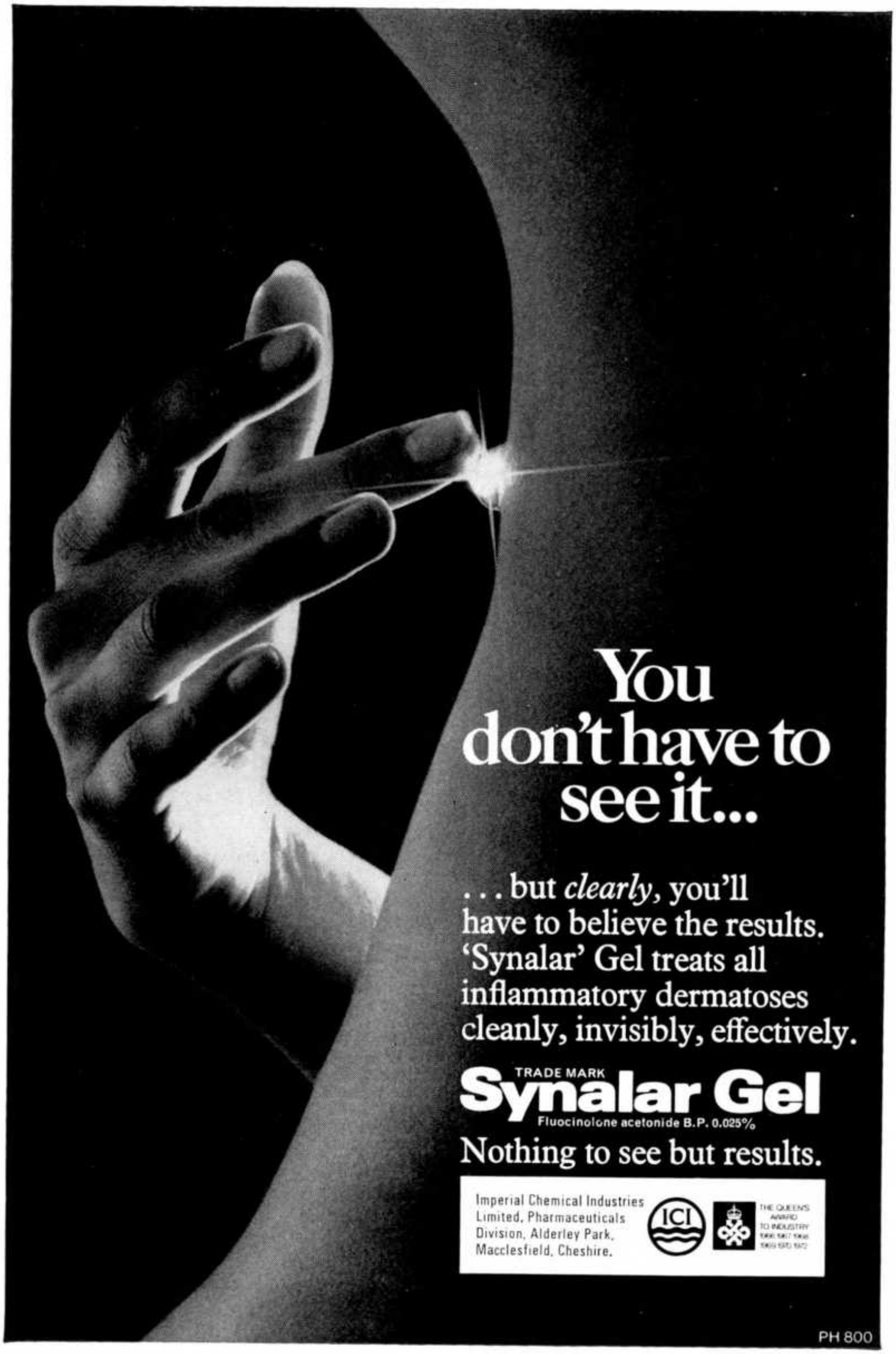

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British Journal of Dermatology (1973) **88**, 213.

Clinical and Laboratory Investigations

The role of dermal blood vessels in the pathogenesis of malignant atrophic papulosis (Degos' disease)

A STUDY OF TWO CASES USING ENZYME HISTOCHEMICAL, FIBRINOLYTIC, ELECTRON-MICROSCOPICAL AND IMMUNOLOGICAL TECHNIQUES

MARTIN M.BLACK, KIYOSHI NISHIOKA* AND GERALD M.LEVENE

Department of Histopathology, Institute of Dermatology, London WC2H 7BJ, and
Department of Immunology, Institute of Dermatology, Homerton Grove, London E9 6BX

Accepted for publication 17 August 1972

SUMMARY

The role of dermal blood vessels in the histopathogenesis of malignant atrophic papulosis has been investigated in two patients using enzyme histochemical, tissue fibrinolysis and electron-microscopic techniques. In the earliest papules the normal enzymatic reactions of the endothelium and fibrinolytic activity of affected blood vessels were markedly impaired or absent. The endothelial cells were swollen and showed minor degenerative changes, but no cytoplasmic inclusions suggestive of virus particles could be detected. The results of intradermal skin tests and lymphocyte transformation cultures did not suggest the presence of any immunological defect. It is suggested that the skin lesions of malignant atrophic papulosis could be secondary to the slow occlusion of deeper arterioles.

Malignant atrophic papulosis (Köhlmeier-Degos) is often a lethal multi-system disease, characterized by the development of typical skin lesions and multiple gastro-intestinal infarctions. The clinical and histological appearances in malignant atrophic papulosis are so distinctive that it is likely that a special histopathogenesis is implicated. We have therefore studied two cases of malignant atrophic papulosis, in whom the lesions were confined to the skin, using enzyme histochemical, tissue fibrinolysis and electron-microscopic techniques. A number of immunological investigations were also performed to see if any immunological defect could be detected.

CASE REPORTS

Case 1

G.W.†, a 29-year-old male first noticed a small papule on the volar aspect of the right wrist 2 years ago. Since then new lesions have continued to erupt on the trunk and extremities. The lesions had been observed to begin as small symptomless papules which later developed white centres. His general health has remained excellent throughout. Examination revealed about thirty discrete lesions in various stages of development scattered over the surface of the trunk and extremities, but sparing the face, scalp and mucous membranes. The early lesions were pale erythematous dermal papules about 3 mm in diameter. The later lesions all had atrophic porcelain-white centres with a sharply defined erythematous halo and did not exceed 0·5 cm in size (Fig. 1). There was no lymphadenopathy, hepato-splenomegaly or abdominal tenderness. No abnormal signs could be detected in the cardiovascular, respiratory or central nervous systems.

---

* Present address: Department of Dermatology, Kansai Medical School, Osaka, Japan.

† Shown at the clinical meeting of the Royal Society of Medicine (Dermatology Section) 21 January 1971 (Black, 1971).

Investigations

The following were normal or negative: Hb, WBC, differential, ESR, urine, blood urea, electrolytes, creatinine, stools for occult blood, plasma proteins, liver function tests, ANF, LE cells, chest X-ray, ECG, papule culture for viruses. Euglobulin lysis time, 3¼ h (prolonged).

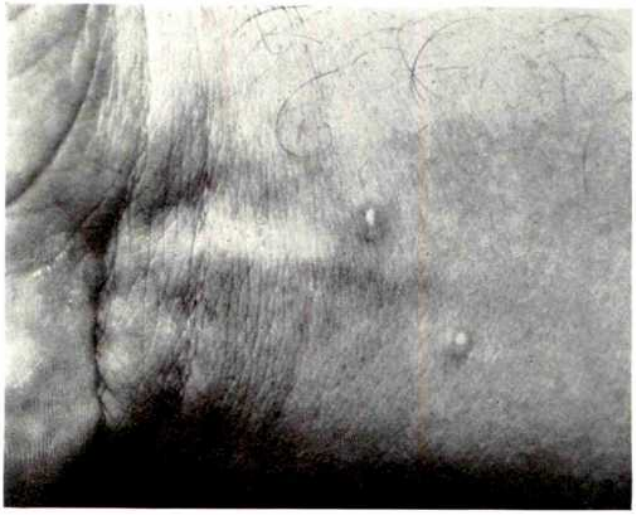

FIGURE 1. Case 1. Two established Degos papules on the volar aspect of the wrist.

Case 2

R.H., a 47-year-old male developed a symptomless papular eruption on the chest 18 months ago. Since then lesions have appeared elsewhere on the trunk and limbs. His general health has been excellent throughout.

Examination revealed a number of typical Degos papules scattered over the trunk and extremities. The early lesions were about 3 mm in diameter, but the fully developed papules with a white porcelain centre were up to 0.5 cm in size. No abnormal signs could be detected on general examination.

Investigations

The following were normal or negative: Hb, WBC, differential, ESR, urine, glucose tolerance test, blood urea, electrolytes, creatinine clearance, stools for occult blood, plasma proteins, liver function tests, ANF, LE cells, chest X-ray, ECG, papule culture for viruses; complement fixing antibody titres to herpesvirus hominis, varicella, vaccinia, enteroviruses, rubella and measles virus. Euglobulin lysis time, greater than 6 h (prolonged).

MATERIALS AND METHODS

Histochemistry

Biopsies were obtained from both the early and established papules. The tissues were frozen immediately by immersion into liquid nitrogen and prepared for histochemistry by sectioning in a cryostat at −20° C.

The following histochemical methods were used:

(1) Toluidine blue stain, for demonstrating the presence of mucin.
(2) Hydrolytic enzymes:
  (a) Non-specific esterase (Holt's indoxyl acetate method, 1954).
  (b) Acid phosphatase (Gomori's lead method, 1941).
  (c) Alkaline phosphatase (azo-dye method of Bancroft, 1967).
  (d) Adenosine triphosphatase (ATPase; lead method after Wachstein, Meisel & Niedzwiedz, 1960).
(3) Respiratory enzymes:
  (a) Succinic dehydrogenase (MTT method of Pearse, 1960).
  (b) Nicotine adenine dinucleotide diaphorase (NAD diaphorase; MTT method of Pearse, 1960).
  (c) Cytochrome oxidase (method of Burstone, 1959).

Fibrinolysis autographs

Frozen sections (8 μm thickness) were applied to fibrin films by the method of Todd (1964) as modified by Turner & Ryan (1969) and incubated at 37° C for 20 min. In the presence of fibrinolytic activity, clear punched-out areas appeared in the opaque fibrin film, usually within 20 min. In the absence of fibrinolysis, incubation was continued at 10 min intervals for up to 60 min. The sections were then fixed in formaldehyde and stained with Harris's haematoxylin.

Electron microscopy

Biopsies were taken from early papules in each patient. Specimens were sliced into small blocks (1–2 mm^3), fixed and embedded in Epon 812. Ultratome sections were stained with uranyl acetate and lead citrate and were examined on an AEI EM6B electron microscope.

RESULTS

Enzyme histochemistry

(1) *Toluidine blue.* Considerable metachromasia was present in the early papules, thus confirming the accumulation of mucin in the centre of the lesions. In the established lesions mucin could only be demonstrated at the margins of the central necrobiotic area where there was a moderately dense perivascular round cell infiltrate.

(2) *Hydrolytic enzymes (non-specific esterase; acid phosphatase; alkaline phosphatase; ATPase).* In the centre of both early and established papules complete loss of the normal reactivity in small blood vessels was found in the centre of the lesions. This area of absent enzyme reactivity was roughly wedge-shaped and extended to the level of the lower dermis. At the margins of the papules the small

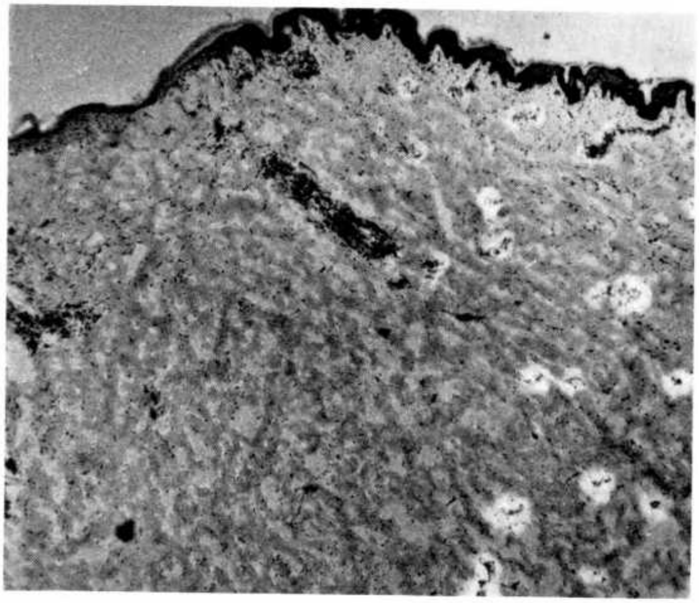

FIGURE 2. Fibrinolysis autograph of established Degos papule (× 20). No fibrinolytic acitivity is evident in the centre of the lesion (on the left), but normal activity is present around small blood vessels, at the edge of the lesion.

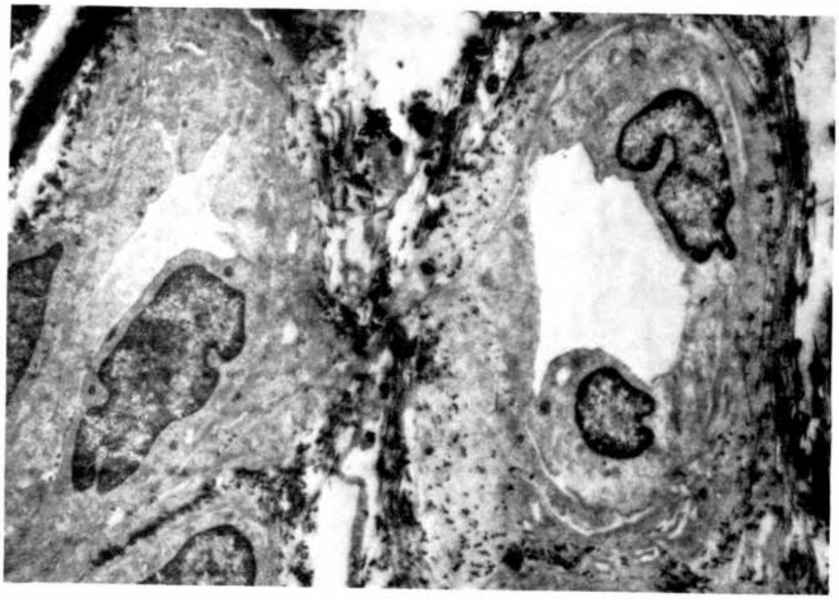

FIGURE 3. The endothelial cells of two small blood vessels appear rather swollen, with some reduction in the size of their lumen (× 2000).

blood vessels showed an increase in activity with the non-specific esterase, acid phosphatase and ATPase enzymes.

(3) *Respiratory enzymes (succinic dehydrogenase; NAD diaphorase)*. The respiratory enzymes also showed complete loss of reactivity in the small blood vessels in the centre of the papules. In the established lesions a decrease in activity was found in the epidermis overlying the centre of the papules.

Fibrinolysis autographs

Complete loss of fibrinolysis was found in the centre of both the early and established papules. The area of absent fibrinolysis was wedge-shaped and extended to the level of the lower dermis (Fig. 2). Normal fibrinolysis was found around small blood vessels at the margins of the papules.

Electron microscopy

In the centre of the earliest papules the endothelial cells of the small blood vessels were rather swollen so that the lumen appeared to be narrowed (Fig. 3). The plasma membrane lining the endothelial cells was present but the extracellular space around the vessels was widened and contained a fine granular material. The cytoplasm of the endothelial cells contained few mitochondria and lysosomes and appeared rather granular and electron dense. However, the microvilli projecting into the lumen were normally present and numerous pinocytic vesicles were also found along the luminal surface of the endothelial cells. No inflammatory cells were found in the vicinity of the affected small vessels and no cytoplasmic inclusions suggestive of virus particles could be detected.

IMMUNOLOGICAL STUDIES

Case 1 was skin tested with Bencard bacterial antigens groups F1 to F5 and to other antigens, and direct immunofluorescent staining with anti-immunoglobulins was carried out on an early lesion (Dr J.J.Cream). Both cases had serum immunoglobulin estimations and in both peripheral blood lymphocyte cultures were prepared (Levene *et al.*, 1969) to assess their response to phytohaemagglutinin (PHA) and to common antigens. No unusual reactions in the immunological studies were found and the results of the tests performed were essentially normal.

DISCUSSION

In malignant atrophic papulosis the small blood vessels are conspicuously involved in the disease process and may be occluded by acellular thrombi. Unlike allergic vasculitis or polyarteritis nodosa the elastic lamina of affected small arteries remains intact and there is no inflammatory response in or around the vessel wall. Furthermore, the time course of the disease and the lack of response to treatment with systemic corticosteroids suggest that malignant atrophic papulosis is distinct from 'allergic' vasculitis or polyarteritis nodosa. Roenigk & Farmer (1968) found raised circulating levels of fibrinogen and cryoprofibrin in two patients with malignant atrophic papulosis and suggested that intravascular coagulation could be a possible factor in the pathogenesis of the disease. Roenigk & Farmer did not investigate the fibrinolytic activity in the serum and skin lesions of their two cases. Here we observed a complete loss of fibrinolysis around small blood vessels in the centre of the earliest papules and in Case 2 the euglobulin lysis time was markedly prolonged. In addition the normal enzymatic reactions of the endothelium in affected blood vessels were also markedly impaired in the earliest skin lesions. These findings suggest a disturbance of endothelial function which leads to an impairment of the normal fibrinolytic activity. On present evidence it is not possible to say whether these changes in endothelial function or fibrinolytic activity could be a primary or secondary manifestation in the histopathogenesis of malignant atrophic papulosis. It is possible, however, that the wedge-shaped involvement of the small blood vessels could arise from the slow occlusion of arterioles situated at a deeper level. Repay (1969) demonstrated the presence of IgG in the damaged vascular wall of a case of malignant atrophic papulosis by direct immunofluorescent staining. However, Jordan, Triftshauser & Schroeter (1971) failed to detect immunoglobulin deposits in two cases of malignant atrophic papulosis and we were also unable to do so in Case 1. The significance of the accumulation of mucin in the skin lesions of malignant atrophic papulosis is unknown, but we have confirmed the findings of Feuerman, Dollberg & Salvador (1970) that mucin is deposited in the earliest papules.

On the basis of a limited number of immunological tests we have failed to find evidence of any immunological defect in our two cases. Roenigk & Farmer (1968) found raised IgA levels in three patients with malignant atrophic papulosis, but in both of our cases the IgA levels were within normal limits.

Howard & Nishida (1969) reported the presence of cytoplasmic inclusions within endothelial cells and suggested that malignant atrophic papulosis may be of viral origin. Like Howard & Nishida we noted swelling of endothelial cells and minor degenerative changes, but we were unable to find any cytoplasmic inclusions which could be interpreted as viruses. No viruses could be cultured from fresh papules and no significant changes in complement fixing antibodies to various viruses were observed. It is suggested therefore that malignant atrophic papulosis is unlikely to be caused by a virus infection.

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The skin in disseminated intravascular coagulation

PROSPECTIVE ANALYSIS OF THIRTY-SIX CASES*

STANLEY J.ROBBOY, MARTIN C.MIHM, ROBERT W.COLMAN AND JOHN D.MINNA

Departments of Pathology, Dermatology and Medicine, Harvard Medical School, and Massachusetts General Hospital, Boston, Department of Pathology, U.S. Ireland Army Hospital, Fort Knox, Kentucky, and the Laboratory of Biochemical Genetics, NHLI, NIH, Bethesda, MD., U.S.A.

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SUMMARY

The cutaneous manifestations of disseminated intravascular coagulation (DIC) present in thirty-three of forty-five patients included petechiae, purpura, palpable variants of both, acral cyanosis, haemorrhagic bullae, purpura fulminans, subcutaneous dissecting haematomata, or bleeding from wound or venipuncture sites. In 47% of cases cutaneous lesions were the presenting sign. Coagulation tests were usually abnormal in a pattern diagnostic of DIC. However when initial coagulation-test observations were equivocal, punch biopsy of the skin yielded fibrin thrombi and thus was helpful in the early diagnosis of DIC, later confirmed by serial coagulation studies. Recognition of the types of skin lesions associated with DIC permits prompt diagnosis and institution of appropriate therapy.

The skin is an important organ in disseminated intravascular coagulation (DIC) (Haim *et al.*, 1970). Cutaneous alterations are frequently the first sign of this disease and their recognition can result in prompt diagnosis and institution of effective therapy. This paper presents clinical and laboratory data on thirty-three patients who had cutaneous signs in thirty-six episodes of DIC and our experience with the skin punch biopsy as a diagnostic tool in confirming the presence of this disseminated process.

METHODS AND MATERIALS

During a prospective study forty-five patients with DIC (Minna, Robboy & Colman, 1973; Robboy, Colman & Minna, 1972), thirty-three patients displayed cutaneous lesions. Three of these patients also experienced a second episode of DIC while in the hospital. The diagnosis of DIC was based on the following tests of coagulation: the platelet count, one-stage prothrombin time, clottable fibrinogen, fibrin(ogen) degradation products (F1, staphylococcal clumping, or tanned red cell haemagglutination

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Requests for reprints: Dr Robboy, Department of Pathology, Massachusetts General Hospital, Boston, Massachusetts 02114 U.S.A.

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inhibition), thrombin time, and euglobulin clot-lysis time (see Table 1 for normal values). The justification for the use of these particular tests is presented elsewhere (Colman, Robboy & Minna, 1972). Heparin (calculated in USP units) was always given intravenously every 4 h.

CASE REPORTS

Case 1

A 39-year-old Caucasoid woman was admitted because of purpura. Six days previously myalgia, arthralgia, and rhinitis appeared, followed by a 39·5°C fever. On the morning of admission multiple purpura appeared on the palms of the hands. Within hours much more purpura developed and she was transferred to the Massachusetts General Hospital. At admission the blood pressure was 50/10 mmHg, pulse 144 and respirations 16. Petechiae and purpura were diffuse, affecting even the conjunctiva. The extremities were most severely involved while the trunk was relatively spared. Many lesions were flat, but some were raised (palpable purpura); some had clear and others necrotic centres. The extremities, especially the fingers, feet, and nose, displayed 3–4 cm patches of cyanosis characterized by a central gun-metal grey colour and erythematous, purplish borders. Initially the lesions were neither pruritic nor painful, and did not blanch. Other pertinent findings included intact radial and dorsalis pedis artery pulses, coarse inspiratory rales at both lung bases, and 50 ml of blood in the vagina. Chest X-ray revealed a diffuse infiltrate in the right lower lobe. Cultures of spinal fluid grew *Neisseria meningitidis*. Because the coagulation changes, which included a titre of fibrin(ogen) degradation products of 1:512, were suggestive of DIC, therapy with both penicillin and heparin (55 U/kg weight) were begun. During the first 24 h new purpura erupted over acral regions, leading to confluent purpura of the extremities that evolved into haemorrhagic bullae. Because the vaginal bleeding persisted the uterus was curetted, but only secretory endometrium was found. By the second hospital day the patient was dramatically improved. The numerous purpuric and other skin lesions began to fade. The haematocrit, which had fallen from an initial value of 39 to 23%, rose to 30%. The prothrombin time fell from 19 s to normal and the fibrinogen rose from 130 to 480 mg/100 ml. The platelet count was unchanged at 36,000/μl. During the ensuing week the purpura that had most recently appeared disappeared. However, some of the purpura in acral regions and especially the large haemorrhagic bullae on the lower leg and arm became extremely tender and gangrenous. On the eighth day heparin therapy was discontinued. By the end of the second week several areas over the left forearm showed deep necrosis and the tips of all but one toe had dry gangrene. She was discharged on the thirty-seventh hospital day; 2 months later the tips of the toes were surgically amputated.

Case 2

A 51-year-old Caucasoid woman was admitted with shaking chills and a 39·5° C fever. Examination revealed a respiratory rate of 30, rales diffusely audible over the chest, and egophony at the right base. A chest X-ray disclosed a massive right lower lobe pneumonia; the sputum contained a pure culture of *Staphylococcus aureus*. Oxacillin and kanamycin were given i.v. During the next 2 days both lungs became markedly opacified on chest X-ray and microabscesses were present in the right lower lobe. A left radial artery catheter (for monitoring blood gases) and a central venous pressure line were placed. On the third day the blood pressure dropped to 90/60 and metaraminol was given i.v. Despite 100% oxygen, the $P\text{O}_2$ never rose above 40 mmHg. Late that day the blood pressure fell further to 60 systolic and an electrocardiogram revealed inferolateral subendocardial ischemia. On the seventh day respirations suddenly became laboured; chest X-ray disclosed a massive pneumothorax and diffuse abscesses. At this time the fingers of the left hand became markedly cyanotic, despite palpable radial artery pulses. Within a day the fingers of both hands (but especially the left), and then several toes, became ecchymotic and gangrenous. Compared with the coagulation-test results taken 3 days previously, the prothrombin time had risen from 12·6 to 23 s, the platelet count had fallen from 186,000 to 95,000/μl, and fibrinogen had fallen from 400 to 140 mg/100 ml. The titre of fibrin(ogen) degradation products was 1:128 and the thrombin time 35 s. There was no other bleeding. Heparin (170 U/kg weight, 8400 U), gentamicin, and chloramphenicol therapy were begun. Despite increasing respiratory distress and an enlarging right pneumothorax, the skin lesions had stabilized 24 h later. On the ninth day the prothrombin time was 19 s and the platelet count 185,000/μl. Three days later the dosage of heparin was reduced (to 120 U/kg weight, 6200 U) and then stopped. On the thirteenth day she had respiratory distress and was hypoxic (Po_2 64 mmHg); tracheostomy was performed. One day later, blood began to ooze from the tracheostomy site and the heparin therapy was restarted. The necrotic skin of the gangrenous left fingers began to slough (Fig. 1). Death occurred on the next day.

Autopsy revealed massive *S. aureus* bronchopneumonia with multiple abscesses, fibrin thrombi in small vessels of both hands and right foot and renal glomerular capillaries, thrombosis of the left radial artery (about cannula), right subclavian vein (about catheter), and left popliteal vein, and interstitital pneumonitis (oxygen toxicity) with extensive alveolar fibrin membranes.

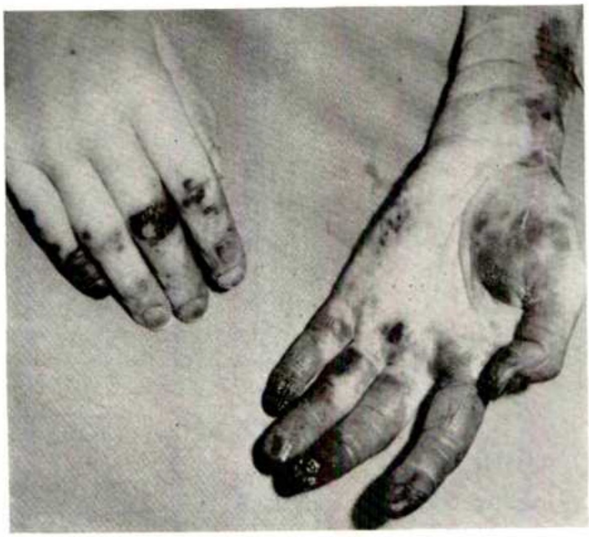

FIGURE 1. Acral cyanosis with haemorrhagic bullae and gangrene in DIC. The irregular borders (so called 'geographic configuration') are sharply demarcated from the uninvolved skin.

RESULTS

The patients ranged in age from 11 to 81 years; half were female and all but one were Caucasian. Tables 1 and 2 present the pertinent coagulation and clinical data. The most common infecting organism was *N. meningitidis*, being isolated in seven cases. Nine other organisms were isolated in an additional ten cases. Acute granulocytic leukaemias and prostatic carcinoma were the most frequently encountered neoplasms (eleven cases). The coagulation changes during DIC consisted of a low platelet count and fibrinogen levels, and prolonged prothrombin time. Tests of fibrinolysis (thrombin time, fibrin(ogen) degradation products, and euglobulin clot lysis time) were positive in all cases (Table 1).

In patients who were in the hospital when DIC developed, serial coagulation tests disclosed falling platelet counts and fibrinogen levels and lengthening of the prothrombin time.

The variety of clinical lesions included petechiae, purpura, palpable purpura, haemorrhagic bullae, purpura fulminans and acral cyanosis. Dissecting subcutaneous haematomas and profuse bleeding from wound and venipuncture sites have a pathogenesis different from the above lesions in that they result from depleted coagulation factors and fibrinolysis rather than from fibrin thrombi or thrombocytopaenia. They have been tabulated because of their identification with the integument, but only when the other skin lesions enumerated above were present. The petechiae were minute, sometimes pinpoint-sized, flat lesions caused by haemorrhage into the skin. Purpura was similar to petechiae, but larger. Raised petechiae and purpura were called palpable petechiae or purpura, respectively. Haemorrhagic bullae were blisters filled with blood. Purpura fulminans, as used in this series, means an extensive confluent purpura of explosive onset that frequently was associated with haemorrhagic bulla formation and focal gangrene. Acral cyanosis referred to gun-metal grey to purplish discolorations of the digits and occasionally, ears or nose that did not blanch with pressure; in these cases the large arterial pulses of the involved extremities were normal. These lesions had irregular borders that were often sharply demarcated from the uninvolved skin (Fig. 1). Dissecting haematomas were extensive subcutaneous accumulations of blood that appeared rapidly as progressive, painful tumefactions.

TABLE 1. Coagulation data in DIC

Tests	Normal (± 1SD)†	Increment of changes as DIC occurs (n = 17)	During DIC (n = 36)	Increment of changes after therapy*	
				Alive or improved (n = 18)	Not improved (n = 11)
Haematocrit	37–50	↓9	30	—	—
Platelet count	250,000	↓120,000	54,000	↑29,000‡	↑14,000
(µl)	± 50,000	± 82,000	± 50,000	± 52,000	± 33,000
Prothrombin time	12·0	↑5·2	18·1	↓3·4	↓0·8
(s)	± 1·0				
Fibrinogen	230	↓251	135	↑184	↑78
(mg/100 ml)	± 35	± 153	± 86	± 124	± 81
FDP titre	1:2	—	1:64 (n = 27)	↓ × 16§	↑ × 2§ (n = 2)
	(0–1:5)				
Euglobulin clot lysis time (min)	> 120		38% abnormal (n = 26)	→normal (n = 6)	→more abnormal (n = 1)
Thrombin time	20	—	29 (n = 19)	—	—
(s)	± 1·6				

* Excludes patients dying within 24 h of initiation of therapy. Data for each test calculated at time patient deemed improved or not improved. In many instances, especially the platelet count, the values returned towards normal after additional periods of time.

† Standard deviation of observations.

‡ Excludes two cases where the increment of change was so great as to appreciably alter the mean. Inclusion gives 68,000 ± 123,000/µl.

§ Changes increase or decrease by factors, thus 1:64 ↓ × 16 = 1:4 and 1:64 ↑ × 2 = 1:128.

The cutaneous manifestations of DIC were observed in thirty-six episodes (thirty-three patients).

TABLE 2. Clinical manifestations

Disease	Number of cases	Initial DIC symptoms related to skin	Petechiae	Purpura	Palpable purpura	Haemorrhagic bullae	Acral cyanosis	Gangrene	Wound venipuncture bleeding	Sub-cutaneous haematoma
Meningococcaemia	6	6	5	6	5	3	2	1	1	0
Gram-negative septicaemia	3	1	2	2	0	1	1	1	1	1
Gram-positive septicaemia	4	2	3	3	1	0	2	1	1	0
Viral infection	2	2	2	1	0	0	1	0	3	0
Aspergillus septicaemia	2	1	2	2	0	0	0	0	1	2
Hypoxaemia and/or hypotension	3	0	2	1	0	0	1	0	3	3
Acute granulocytic leukaemia	7	1	5	7	1	1	0	0	4	2
Epithelial carcinoma	5	2	0	5	1	1	1	1	2	1
Amniotic fluid embolism	1	0	0	0	0	0	1	0	0	0
Purpura fulminans	2	2	1	2	2	1	0	2	0	2
Fulminant hepatitis	1	0	0	1	0	0	0	0	0	0
Series	36	47%	61%	83%	32%	19%	25%	17%	39%	31%

In 47% of these episodes the skin lesions prompted the patient to seek medical advice and heralded the onset of DIC. In general the presenting cutaneous lesions were petechiae (58%), purpura (31%), palpable petechiae–purpura (19%), or acral cyanosis (11%), each of which evolved during a several hour to 3 day period. Usually the petechiae and purpura remained confined to the extremities. At times they spread in a centripetal fashion, or were generalized. On occasion (19%) the purpura progressed to haemorrhagic bullae, and in two patients into purpura fulminans. Sometimes, especially in meningococcaemia, the petechiae and purpura were palpable. Palpable petechiae and purpura were encountered most commonly with septicaemia and purpura fulminans.

In four patients acral cyanosis evolved into gangrene of the acral regions. The presence of either indwelling arterial or venous catheters in an involved extremity was associated with more extensive acral cyanosis and gangrenous complications. In two cases, nasal gangrene was also observed. Not infrequently patients with acral cyanosis who improved had gangrenous digital tips that self-amputated or eventually required surgical amputation. This occurred in three patients who are alive today, and would have occurred in two others (Fig. 1) had they survived to leave the hospital.

53% of the patients were already in the hospital when the mechanism that triggered DIC occurred. In most of these cases the skin manifestation appeared only after there was other clinical (e.g. haemoptysis, haematemesis, shock) or laboratory evidence of DIC. The interval was several hours to more than 7 days (1–2 day mean) before the skin manifestation developed. Only five of the patients presented with cutaneous lesions, three of whom had acral cyanosis and one of whom had re-exacerbation of purpura fulminans. The patients developed their DIC because of nosocomial acquired infections, periods of prolonged hypoxia or hypotension, or typically 3–6 days after chemotherapy was given for neoplasia (in the last named situation the result of tissue thromboplastin liberated from damaged cells may explain the mechanism by which DIC occurs after chemotherapy) (Colman, Robboy & Minna, 1972).

The response of patients to a combination of heparin therapy (45 to rarely above 135 USP units/kg body weight every 4 h) and specific therapy directed to the underlying disease (e.g. antibiotics for sepsis) was usually evident within 1–4 days. Improvement was scored when new lesions ceased appearing, lesions already present began resolving, and coagulation tests began improving (50% of patients); one-third of the improved patients subsequently died of the basic underlying disease processes. Failure to improve was associated with new cutaneous lesions, exacerbation of lesions already present, or negligible or deteriorating coagulation changes (31%); these patients usually died. The remaining 19% of patients died less than 24 h after diagnosis of DIC and were considered ineligible for evaluation of response to therapy.

Pathology

Fibrin thrombi in small vessels of the skin, were found in fifteen of twenty-three cases, including the five of seven cases where biopsy was performed as a diagnostic procedure. This frequency rate (65%) was second only to the kidney (68%). In petechiae or purpura present for less than 24 h, fibrin thrombi were observed in the capillaries of the superficial vascular plexus which lies approximately at the junction of the papillary and reticular dermis. The tightly woven, eosinophilic fibrin aggregates were usually located centrally in the vascular lumina and only rarely abutted on the vascular walls. Extravasation of red blood cells was present, but little or no perivascular inflammatory infiltrate was observed. Biopsies of older lesions (2–3 days) revealed epidermal necrosis, formation of subepidermal bullae, and patchy necrosis of the eccrine glands and pilosebaceous apparatus (Fig. 2). Lesions that clinically progressed to gangrene showed extensive necrosis of the epidermis, dermis and cutaneous appendages.

An inflammatory infiltrate was observed only rarely in the dermis, consisting of occasional lymphocytes and polymorphonuclear leukocytes. Gangrenous lesions obtained by amputation revealed full digital necrosis, including the bone.

The skin was examined microscopically in nine cases of palpable purpura. In all but two, fibrin thrombi in small vessels and extensive haemorrhage were observed. No cases of allergic cutaneous vasculitis were found.

At autopsy fibrin thrombi were found in at least one organ in all but one of the sixteen autopsies. These thrombi were sometimes inconspicuous in histological tissue sections and on occasion were present only in a single organ. Many organs and tissues contained haemorrhages or haematomas; none, however, displayed histologic evidence of vasculitis, a condition characterized by inflammatory changes in the vessel wall. Twenty-six photographs depicting the skin and general autopsy changes in DIC, as well as a discussion about the specificity and differential diagnosis of fibrin thrombi are presented elsewhere (Robboy *et al.*, 1972).

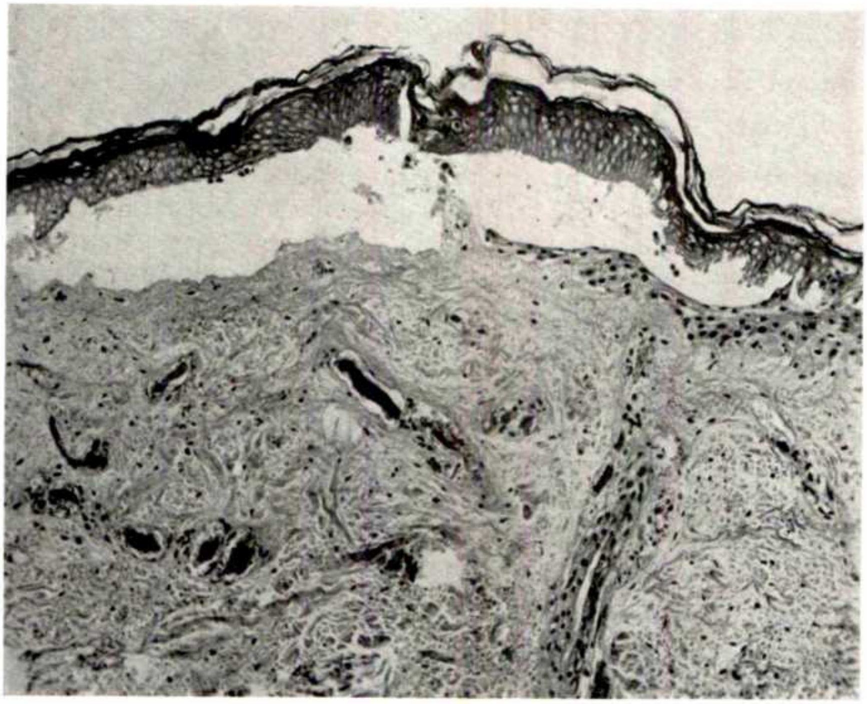

FIGURE 2. Subepidermal bulla with necrotic epidermis covering bulla, nucleated squamous cells at margin of lesion (right), and fibrin thrombi plugging small venules (H & E, × 140).

DISCUSSION

What skin findings can be of aid in the early diagnosis of DIC? Petechiae or purpura are found in many haemorrhagic disorders besides DIC, and their appearance usually leads to appropriate haematological evaluation. The clinical challenge arises when the initial signs are palpable petechiae, palpable purpura, haemorrhagic bullae, or acral cyanosis—lesions that are not widely recognized as within the symptom complex of DIC, but whose nature cutaneous biopsy can disclose.

The syndrome of septic shock, palpable purpura, and/or acral cyanosis is highly suggestive of

DIC. Other causes of palpable purpura are septic embolic lesions (e.g. subacute bacterial endocarditis) and allergic cutaneous vasculitis, a symptom complex that may occur as an idiopathic disorder confined to the skin, an adverse reaction to drugs, frequently penicillin or sulphonamides, or during the course of systemic necrotizing angiitis, such as hypersensitivity angiitis (Mills, 1971). The histopathologic findings in allergic cutaneous vasculitis include 'fibrinoid necrosis' of the small vessel wall (apparently venules), intense infiltration of polymorphonuclear leukocytes (PMN) in and about the vessel wall, PMN nuclear debris (so called 'nuclear dust'), and extravasation of red blood cells (Lever, 1967). This picture differs from DIC, where fibrin thrombi fill vessels of varying sizes; the walls may occasionally exhibit necrosis, but not the inflammatory changes seen in vasculitis. Clinically, the two conditions may be difficult to distinguish from each other since in both the patient may appear acutely ill.

Haemorrhagic bullae may occur during the course of any of the subepidermal–bullous disorders. In none of the more common ones, such as erythema multiforme or bullous pemphigoid are there fibrin thrombi in vessels (Lever, 1967). In addition, the latter disorders may be characterized by specific epidermal and dermal inflammatory changes. It should be stressed that should septicaemia or shock intervene, DIC may develop in addition to the allergic cutaneous vasculitis or subepidermal bullous disorders.

The association of acral cyanosis with DIC particularly in the presence of septicaemia, has been noted (Fox, 1971; Meyers, Hirschman & Sloan, 1970). Most patients are acutely and seriously ill, hypotensive, and have multiple signs of impaired tissue perfusion (e.g. reduced renal function). Although some patients also have acute pulmonary (e.g. bronchopneumonia) or cardiac disease, their acral and facial cyanosis appears out of proportion to the degree of hypoxaemia. The acral cyanosis of DIC is gun-metal grey, sharply demarcated, and symmetrical, and the tips of the digits or nose may be blue-black, suggesting impending gangrene. Pulsations in arteries are intact (except when arterial catheters are present), which rules out embolic lesions; the capillary perfusion is grossly decreased, even when the skin is warm, reflecting the occlusive nature of the fibrin thrombi in small vessels. These characteristics plus the clinical setting of sepsis, shock, or neoplasia, make the diagnosis of the acral cyanosis in the more indolent diseases, Raynaud's phenomenon, chilblains, or acrocyanosis, unlikely (Spittell, 1972).

Once the possibility of DIC is entertained because of clinical manifestations (bleeding, thrombosis, acral cyanosis) or disease settings (e.g. septic shock), the diagnosis can be confirmed in the large majority of instances by simple coagulation tests. The triad of prolonged prothrombin time, thrombocytopaenia, and hypofibrinogenaemia is highly reliable if not pathognomonic for DIC (Minna *et al.*, 1973). Patients with this triad also have positive tests for fibrinolysis. A point of confusion arises when patients simultaneously have liver disease. By using quantitatively more rigid criteria the same coagulation tests can be used to identify which of these patients also have DIC (Minna *et al.*, 1973). If the clinical setting suggests DIC but the laboratory tests are equivocal, skin biopsy (especially of palpable purpura, haemorrhagic bullae, or acral cyanosis) and examination for fibrin thrombi is a useful diagnostic tool. Thus, seven of nine patients had fibrin thrombi demonstrated in this series. The major problem with cutaneous biopsy is the 12–24 h interval required to process the specimen, thus delaying the time before diagnosis is established and therapy instituted.

Therapy of patients with DIC is not directed towards the skin, but to (1) control of the underlying disease (e.g. sepsis, hypoxaemia, acidosis, removal of precipitating factors); (2) correction of other haemostatic abnormalities (e.g. vitamin deficiency), and (3) consideration of heparin therapy. Although the role of heparin in DIC is controversial, our data suggests that heparin is an effective and safe drug in treating clinically significant DIC (Colman *et al.*, 1973, Minna *et al.*, 1973). The dosage schedule we prefer is 85 USP units/kg body weight every 4 h intravenously. Bleeding may increase slightly during the first 24 h, but appears to result from yet unchecked DIC, rather than as a complication of therapy. Most of our patients who improved (defined as complete cessation of bleeding and return towards normal of coagulation test results) did so in 1–5 days (average 3). Sometimes heparin was given for 1 week or more before a cure was evident. On occasion a resistance to heparin was experienced (increased bleeding and deteriorating coagulation test results) either when therapy was begun or when a second episode of DIC occurred. In these cases the dosage of heparin was temporarily raised as high as 135 USP units/kg body weight every 4 h. With vigorous heparin therapy, DIC frequently abated, even though many of these patients never survived to leave the hospital because of the underlying disease processes (e.g. leukaemia). The resolution or progression of the cutaneous manifestations of DIC are good indicators respectively of a favourable response to therapy or of relapse.

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Observations on tissue fibrinolytic activity in patients with cutaneous vasculitis

B.DODMAN, W.J.CUNLIFFE AND B.E.ROBERTS

Department of Dermatology, Department of Haematology, The General Infirmary, Leeds
St James's (University) Hospital, Leeds, and Harrogate General Hospital

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SUMMARY

Skin fibrinolytic activity was measured in the normal looking skin of the arms and legs of patients with vasculitis and in control subjects. In both groups the tissue activity was lower in the legs than arms and this may be one of the pathogenetic features localizing certain vasculitic lesions to the legs.

The fibrinolytic activity was almost absent in the vasculitic lesion but it was also significantly lower in the normal looking skin of the patients compared to controls. It is suggested that this might represent utilization of fibrinolytic enzymes in the patient's skin thereby contributing to the perpetuation of the rash.

The role of fibrinolysis in cutaneous vasculitis is controversial. Some authors (Kurban & Ryan, 1969; Vaithaininathan & Takats 1968) have found no difference in blood fibrinolytic activity in patients with cutaneous vasculitis whereas others (Cunliffe, 1968; Cunliffe & Menon, 1971; Isacson *et al.*, 1970) have demonstrated that some patients with a cutaneous vasculitis have a significantly reduced blood fibrinolytic activity. It might, however, be more meaningful to look at skin rather than blood fibrinolytic activity.

MATERIALS AND METHODS

We investigated thirty-two subjects with minimal skin lesions (aged 17–77, mean 39 ± 17) and twenty-eight patients with cutaneous vasculitis, the clinical details of which are shown in Table 1.

Collateral observations (Dodman *et al.*, 1972) have shown that obesity, age and sex probably do not significantly affect skin fibrinolytic activity. However, to minimize any other possible variables we took the biopsies from the subjects between 9.00 and 11.00 a.m., the patients fasting, resting and not taking any medication.

The biopsies were taken from the centre of an already developed vasculitic lesion and from macroscopically normal sites on the mid-forearm (medially) and the mid-calf (laterally) in all subjects. The samples were processed using Turner & Ryan's (1969) modification of Todd's technique (1959). One of the problems of tissue fibrinolysis is the lack of quantitation in recording the results. We used a technique previously described (Cunliffe *et al.*, 1971; Dodman *et al.*, 1972) in which we record the

TABLE 1. The clinical details of the patients

Sex	*Age*	*Diagnosis*
F	25	Nodular vasculitis
F	29	Nodular vasculitis
F	54	Nodular vasculitis
F	46	Nodular vasculitis
F	46	Nodular vasculitis
F	59	Nodular vasculitis
F	32	Erythema nodosum
F	45	Erythema nodosum
F	43	Erythema nodosum
F	47	Erythema nodosum
M	50	Purpuric vasculitis
F	66	Purpuric vasculitis
F	30	Purpuric vasculitis
F	46	Purpuric vasculitis
M	67	Purpuric vasculitis
M	46	Purpuric vasculitis
M	69	Purpuric vasculitis
F	67	Purpuric vasculitis
M	51	Rheumatoid vasculitis
M	43	Rheumatoid vasculitis
M	32	Pityriasis lichenoides chronica
F	16	Pityriasis lichenoides chronica
F	43	Behcet's syndrome
F	32	Behcet's syndrome
M	36	Behcet's syndrome
M	55	Panniculitis
F	45	Erythema induratum
F	60	Henoch Schönlein purpura

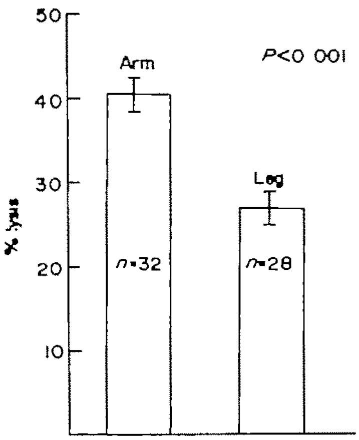

FIGURE 1. The tissue activity in control subjects.

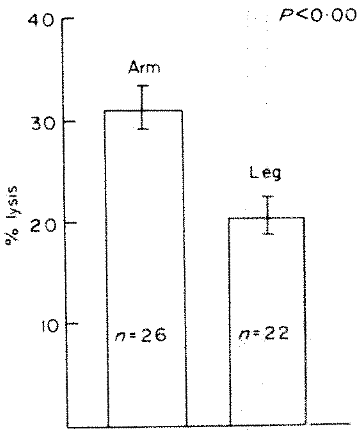

FIGURE 2. The tissue activity in patients with vasculitis (normal looking skin).

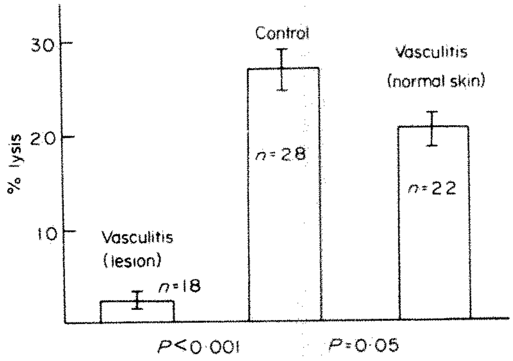

FIGURE 3. The tissue activity in the arms of patients compared to controls.

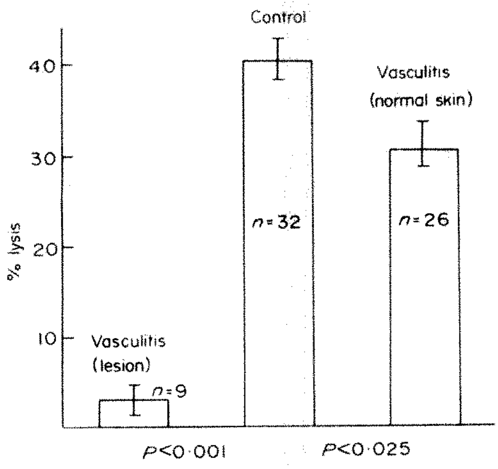

FIGURE 4. The tissue activity in the legs of patients compared to controls.

area of lysis as a percentage of the whole section. Using a 16 point grid graticule in the eye piece we recorded a 'hit' each time an intersection of the graticule overlay an area of lysis. We positioned each section on the stage of the microscope in a series of positions previously determined using computer generated random number tables (Dodman *et al.*, 1972). This makes the results statistically valid at the 5% level. We also recorded the number and size of vessels using the same technique.

RESULTS

Figures 1 and 2 show that the tissue fibrinolytic activity is significantly lower in the legs as compared to the arms in both the controls and the patients. Although there is a highly significant reduction of the activity in a vasculitic lesion there is also a significant reduction in fibrinolytic activity in the normal looking skin of both arm and leg of the patient as compared to control data (Figs. 3 and 4). No difference was seen between the number and size of blood vessels in a standard area of arm and leg skin (Table 2).

TABLE 2. The number of vessels per unit area (mean ± SEM). The difference is not statistically significant ($P = 0{\cdot}1$)

Arm vessels	Leg vessels
18 ± 0·67 $n = 46$	16·3 ± 0·63 $n = 42$

DISCUSSION

The reduced fibrinolytic activity in the normal looking leg skin is in keeping with the observations of Pandolfi *et al.* (1967) of a reduced fibrinolytic activity in leg veins and venous leg blood as compared with that of the arms. Our data demonstrates that this decrease is not related either to fewer or smaller blood vessels in the leg. These differences between arm and leg may simply represent differences in the structure and function of the venules and veins at these sites. In the arm the veins and venules are more cullular whereas in the leg there are considerably greater amounts of elastic tissue (Pandolfi *et al.*, 1967). The more cellular structure of the arm vessels is a better source of plasminogen activator which is one of the limiting factors in fibrinolytic mechanisms. It is suggested that the decreased fibrinolytic activity in the leg could possibly be one of the pathogenetic factors localizing many cutaneous vasculitic lesions to the lower leg.

Our finding of a reduced tissue activity particularly in the lesions does not necessarily imply a reduced plasminogen activator in the tissues; the decrease could also be due to an increase of fibrinolytic inhibitors or binding of the fibrinolytic enzymes. Inflammatory lesions such as psoriasis and eczema (Dodman & Cunliffe, 1972) have shown a minimal reduction of skin fibrinolysis. It therefore seems unlikely that the decrease is specifically related to a particular type of cellular infiltrate and investigations on the relationship between reduced fibrinolytic activity and the cellular infiltrate are underway.

Since fibrinolysis is a physiological process to remove fibrin, the marked reduction in the lesions could simply represent utilization of the fibrinolytic enzymes to prevent further sludging and thrombus formation. The reduced levels in the skin away from the lesions are more difficult to explain;

although unlikely, they may indicate mobilization of plasminogen activator enzyme to the lesions which is usually the limiting factor in reduced fibrinolytic states. Alternatively, they could represent binding of the enzymes to a fibrin film on the walls of the blood vessels.

We conclude that there is a significant reduction in skin fibrinolytic activity in certain patients with a cutaneous vasculitis. These observations support the role of impaired fibrinolytic mechanisms in patients with vasculitis and need to be extended in a study of tissue fibrinolysis associated with the natural resolution of vasculitis and its treatment with effective fibrinolytic agents (Fearnley, Chakrabasti & Hockling, 1967).

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An improved method for the isolation of fungi in onychomycosis

MARY P. ENGLISH AND R. ATKINSON

Mycology Laboratory, Pathology Department, Royal Infirmary, Bristol BS2 8HW, and
Fishponds Health Centre, Beechwood Road, Bristol 7

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SUMMARY

A description is given of the use of a drill for obtaining nail samples for fungal culture in cases of onychomycosis. The nail dust produced by the drill is collected by suction into a bag, and used to inoculate culture plates. Nail dust was found to be greatly superior to clippings for the successful culture of both dermatophytes and non-dermatophytes. Possible reasons for this are discussed and it is suggested that in dermatological practice a drill should be used for sampling nails where attempts to culture from clippings have failed.

The difficulty of isolating fungi from nail clippings in cases of onychomycosis has long been recognized and has been discussed by many workers. Except in strictly superficial infections of the leukonychia trichophytica type (Jessner, 1922), fungi usually grow most actively at the proximal end of the nail (Gentles, 1971): clippings are necessarily taken from the distal end where the hyphae are often dead, having arrived at this site only by nail growth. Consequently, though hyphae in clippings can be seen on direct microscopy, cultures are frequently unsuccessful.

In 1945, Epstein suggested the use of a dental drill for obtaining specimens from nails for mycological examination, but the method has never become popular. But the use of a drill allows the whole, or any part, of a nail to be sampled to a considerable depth, thus enabling the investigator to reach sites where the fungus is still alive.

One difficulty of sampling by this means is the collection of the dust. This can be overcome by the use of a drill (SUDA Suction Drill, Thomas Holland & Son Ltd, Brewery Road, London N.W.7) (Fig. 1) which sucks the dust, as it is generated, into a disposable bag housed under its motor. Results obtained from the use of such a drill are described below.

PATIENTS AND METHODS

Only toe-nails were studied. All chiropodal work was carried out by R.A. and laboratory studies by M.P.E. The patients were those presenting at two local authority clinics (A and B) in Bristol. These catered for old age pensioners: consequently all women were over 60 years of age and all men over 65. 90% of patients were women. The drill was used only on pathologically thickened nails which would benefit from such treatment.

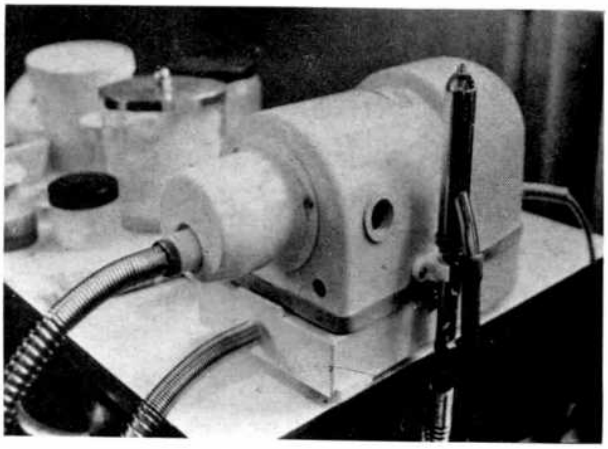

FIGURE 1. SUDA Suction Drill.

The suction power of the drill is such that only the finer particles of dust are drawn into the receiving bag, the larger fragments falling to the floor. An initial study having shown that dust gave better results than the larger particles, a fine burr, which gave the maximum amount of dust, was used throughout. A fresh collecting bag was used for each patient, but it was not possible to sterilize the drill head or suction tube between patients. Clippings, when taken, were obtained before the nails were burred. Nail dust and clippings were examined by direct microscopy in KOH squashes, and were cultured on glucose–peptone–chloramphenicol agar, with and without actidione. Dust was sprinkled over the surface of the agar, and any non-dermatophyte grown was considered to be of clinical significance only if a large number of colonies were produced. Cultures from clippings were made and evaluated as described by Walshe & English (1966).

RESULTS

Preliminary trial

This was carried out on thirty patients seen in clinic A. The results of examination of nail dusts are

TABLE 1. Results of laboratory examination of toe-nail dusts from thirty patients in clinic A

No. of cases	Microscopical result	Cultural result
16	Neg.	Neg.
3	Neg.	*Candida parapsilosis*
2	Pos.	Cultures failed
4	Pos.	*Trichophyton interdigitale*
1	Pos.	*Scopulariopsis brevicaulis*
1	Pos.	*Penicillium pallidum*
1	Pos.	*Aspergillus versicolor*
1	Pos.	*Cephalosporium madurae*
1	Pos.	*Hendersonula toruloidea**

* Patient a Jamaican.

shown in Table 1. Of the eighteen microscopically negative cases, fifteen were also negative on culturing: the remaining three yielded large quantities of *Candida parapsilosis*. Of the eleven microscopically positive cases, nine were successfully cultured.

The exceptionally high proportion of successful cultures obtained from these microscopically positive nails encouraged us to carry out a more detailed study of the technique.

Main study

This was carried out on forty patients seen in clinic B. Clippings were taken from the nails in order to compare the results obtained from them with those from dust. The order in which the patients were treated by the chiropodist was recorded.

Table 2 compares the findings from clippings and from dust. Though the microscopical results from the two types of specimen were identical it was usually found necessary to spend about twice as long searching dust squashes as squashes from clippings before hyphae were seen. In twenty-one patients no evidence of fungal infection was found by any test. Large quantities of *C. parapsilosis* were isolated from the dust from one microscopically negative patient. A second microscopically negative patient yielded very large quantities of *Aspergillus versicolor* in the dust culture and a few colonies of the same fungus from the clippings. Of five patients with dermatophyte infections the fungi were grown from the dust of four and the clippings of two.

TABLE 2. Results of laboratory examination of toe-nail dust and clippings from forty patients in clinic B

Fungus	No. of cases	Microscopical results*	No. of positive cultures	
			Clippings†	Dust
None	22	Neg.	0	0
Candida parapsilosis	1	Neg.	0	1
Trichophyton interdigitale	3	Pos.	1	3
T. rubrum	1	Pos.	1	1
Dermatophyte and *C. albicans*	1	Pos. (dermatophyte and yeast)	*C. albicans* only	*C. albicans* only
Aspergillus versicolor	3	2 pos. 1 neg.	2 (1)	3
A. versicolor *A. candidus* } mixed	1	Pos.	(1)	1
A. terreus	3	Pos.	1 (1)	3
Cephalosporium spp.	3	Pos.	1 (2)	3
S. brevicaulis	2	Pos.	(1)	1

* Results from clippings and dust identical.

† Brackets indicate fungus grown, but from fewer than 5/20 inocula.

In the fifth patient, all of whose cultures failed, the skin of the feet, as well as the nails, was seen on microscopy to be heavily infected: it was therefore concluded that the nail infection was due to a dermatophyte: this patient's nails were also heavily infected with *Candida albicans*, distinguishable from the dermatophyte on direct microscopy and isolated from both dust and clippings.

Eleven microscopically positive patients were infected by non-dermatophytes. Fungi were successfully isolated from the dust cultures of ten. The single case in which cultures failed was one of

Scopulariopsis brevicaulis infection, identifiable by the conidia seen on direct microscopy. Clippings from four of the eleven patients yielded five or more colonies of the appropriate fungus from the twenty inocula used and cultures would, therefore, have been reported as successful by the criteria of Walshe & English (1966). Cultures of clippings from six other patients yielded the same fungi as the dust cultures, but the organisms grew from fewer than five of the twenty inocula: they would, therefore, have been reported as 'cultures failed' without the supporting evidence of the dust cultures. One patient from whose dust culture a very heavy growth of *A. versicolor* was obtained, had nails in which fungi were never found on direct microscopy of either clippings or dust.

To summarize, in the seventeen patients with microscopically positive toe-nails, the causal fungi were successfully isolated from the dusts of fifteen, but (by the criteria of Walshe & English) from the clippings of only six.

A number of microscopically negative dusts from clinic B patients yielded in culture a few colonies of *C. parapsilosis*, *C. guilliermondii* and various non-dermatophytes. The occurrence of the yeasts, both in these cases and in those microscopically negative nails which yielded large quantities of *C. parapsilosis*, needs further investigation. Of the non-dermatophytes, *Cephalosporium* spp. were found in the dusts of two patients, *A. versicolor* in two and *A. terreus* in one. Each of these patients was treated immediately after one who was genuinely infected with the same fungus, *Aspergillus*-type conidial heads having been seen on direct microscopy in one patient with *A. versicolor* infection and one with *A. terreus*. Carry-over of a fungus by means of the unsterilized suction tube of the drill can therefore occur, especially if a non-dermatophyte is sporing *in situ* in the nail. But, because of the few colonies isolated and the negative microscopy, this carry-over could not be confused with a genuine infection. No carry-over of dermatophytes was noted. It seems reasonable to assume that the occasional colonies of other common moulds were air- or nail-borne contaminants.

DISCUSSION AND CONCLUSIONS

Drill dust gives a far higher proportion of successful cultures from microscopically positive toe-nails than do the more conventional clippings. This is almost certainly due to the fact that, with a drill, it is possible to obtain specimens from parts of the nail in which the fungus is active, though the powdered form may also, in some way, encourage fungal growth (Zaias, 1966). It is quicker and easier to handle dust in the laboratory than to chop up clippings finely for microscopy and culture. A disadvantage of dust specimens in the laboratory is that fungal hyphae are more difficult to find on direct microscopy than in clippings, and consequently more time must be spent searching for them. Contamination via the drill of one patient's dust by that of the previous patient is not a serious problem.

Table 3 which combines the results from both clinics, shows that, for dermatophytes and moulds, there is good agreement between microscopy and culture of dusts, both for infected and uninfected nails. Excluding the four cases where *C. parapsilosis* was found, sixty-one of the remaining sixty-six patients (92%) were either positive or negative by both tests. The consistent failure to find *C. parapsilosis* on direct microscopy could be due to the difficulty of finding yeast cells in unstained squashes of dust, for *C. parapsilosis* very rarely forms pseudomycelium *in vivo* (English, Smith & Harman, 1970; English, personal observations on nails.)

The six cases in which clippings yielded the same non-dermatophyte in culture as the dust, but only from a few inocula, might suggest that the arbitrary criteria used by Walshe & English (1966) to diagnose onychomycosis due to a non-dermatophyte may be too stringent. However, in diagnostic practice it is not unusual to grow a few colonies of a non-dermatophyte from microscopically negative nail clippings and also from those clearly infected by a dermatophyte (Zaias, Oertal & Elliott, 1969).

TABLE 3. Agreement between dust cultures and direct microscopy in seventy patients in clinics A and B combined

Microscopy	Culture	
	Positive	Negative
Positive	23	4
Negative	5*	38

* One non-dermatophyte, four *C. parapsilosis*.

When only clippings are available it is therefore probably wise to continue to incriminate a non-dermatophyte only on the basis of the criteria originally proposed.

When sampling nails it is considerably quicker to take clippings than drillings. For this reason, dermatologists using a drill in clinical practice might prefer to take nail clippings in the first place, and only resort to the drill when the laboratory has reported positive microscopy but failed cultures.

The efficiency of other types of drill is being investigated.

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Trichophyton soudanense infection in two white families

Z.HARARI, B.SOMMER AND A.FEINSTEIN

Department of Dermatology, The Chaim Sheba Medical Centre, Tel Hashomer
and Tel Aviv University Medical School, Israel

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SUMMARY

The isolation of *Trichophyton soudanense* outside of Africa has been reported relatively infrequently and in single cases only. As far as we know widespread infection within white families has not previously been noted. We have isolated *T. soudanense* from seven individuals in a family of ten, from three of seven members of a second family, and from a classmate of one of the affected children in the first family.

CASE REPORTS

The interesting chain of events began in January 1972 when our mycological laboratory received a sample of hair from a 13-year-old girl. Direct microscopy revealed endothrix infection but with larger arthrospores than in *T. violaceum*, which is prevalent in Israel. *T. soudanense* was isolated.

We examined the whole family of which the parents and most of the eight children had been born in Tunisia and had migrated to Israel 4 years previously. The mother had suffered for many years, while in Tunisia, from a lesion on her left forearm which had not been treated. The father had been irradiated in childhood because of tinea capitis and was bald on examination. In six (three girls, three boys) of the children nine clinically suspicious lesions (five scalp, two buttocks, one hand, one foot) were detected and we isolated *T. soudanense* from these and from the mother's forearm.

One month later we received a hair sample from a classmate of one of the girls of this family and again isolated *T. soudanense*.

The second family was referred for investigation about the same time because of tinea capitis diagnosed by a local dermatologist. The father had been born in Yemen and had lived in Israel for the past 20 years. We found five suspicious lesions (three scalp, one hand, one neck) in three children (all boys) and isolated *T. soudanense*. The parents and the other two children had no lesions.

There were neither geographical nor familial connections between the two families.

DISCUSSION

The first detailed report of dermatomycoses in Israel (Sagher, 1947) was published before the mass immigration from North Africa which began in 1948. Sagher does not mention *T. soudanense* nor do Feinstein *et al.* (1971) or Ziprkowski (1971) who respectively report almost 10,000 and 50,000 isolations. The first published report of *T. soudanense* in Israel is that by Henig, Portuch-Eisenkraft & Lehrer (1970) in two white immigrants from North Africa, but we learn from a personal communication (Altman, 1972) that this species was isolated from a white immigrant from North Africa in 1961.

Vanbreuseghem (1968) states that every case of *T. soudanense* infection detected outside of Africa comes directly from Africa or is acquired by contact with someone who has been in Africa. Our first family had apparently imported *T. soudanense* from North Africa as the mother had her skin lesion there and most of the children were born in Tunisia. There can be little doubt that the school friend contracted the infection from the 13-year-old girl of this family. The children of the second family had contact at school with children of North African origin and this probably explains this source of their infections.

Calnan, Djavahiszwili & Hodgson (1962) state that it is difficult to know what epidemiological factors determine the spread of fungous infections in communities. Ziprkowski's (1971) large survey shows that *T. soudanense* has not yet become established in Israel but our findings and the cases of Henig *et al.* (1970) indicate that this dermatophyte occurs in white immigrants from North Africa and that infection may be transferred within Israel to others. White immigrants from North Africa represent a considerable proportion of the Israeli population; we may presume that endemic areas exist and that further isolations of *T. soudanense* may be expected.

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Active epidermal movement in human skin *in vitro*

R.MARKS AND T.NISHIKAWA

Department of Experimental Pathology, Institute of Dermatology, and
St John's Hospital for Diseases of the Skin, Leicester Square, London W.C.2

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SUMMARY

Epidermal cells migrate from the edges of adult human skin in culture during the first 48 h. In order to determine whether mitotic acitivity was responsible for this cell movement the autoradiographic labelling index for cells in DNA synthesis was determined for skin at different stages of culture. The labelling index of skin maintained for 48 h but labelled with tritiated thymidine in the first 4 h, did not differ significantly from skin both maintained and labelled for 4 h only. Skin maintained for 48 h but labelled in the last 4 h showed a much lower labelling index. No normal mitotic figures were seen in the epidermis of skin maintained for 48 h but exposed to colcemid for the last 4 h of culture.

It has been concluded that at least in short-term organ culture of adult human skin there is a phase of mitotic arrest and that epidermal migration during the first 48 h is an active process independent of mitotic activity.

The primary epidermal response to injury consists of a complex series of events involving both active movement of epidermal cells and increase in the mitotic rate (Viziam, Matoltsy & Mescon, 1964). Over the years there have been frequent discussions in the literature as to the relative importance of active cell movement and the epidermal proliferative response in epidermal wound healing (Matoltsy, 1955; Viziam *et al.*, 1964; Winter, 1964; Raferty, 1971). We have been particularly interested in the factors that control epidermal cell movement after injury and in order to study this aspect of epithelial behaviour we have used a simple *in vitro* model consisting of fragments of human skin maintained in culture for a 48-h period. During this short period of culture, the epidermis migrates from the edges of the fragment around the free dermal aspect of the explant (Medawar, 1948; Cruickshank, 1965; Sarkany, Grice & Caron, 1965; Marks, Bhogal & Dawber, 1972). In this paper we provide evidence that this migratory activity, in the model that we use, is due to active epidermal cell movement rather than proliferative activity.

METHODS

Normal skin was removed using the Castroviejo keratotome set at 0·4 mm from the thighs of volunteers who had given their informed consent. After removal, the thin sheets of skin obtained were divided into rectangles of approximately 0·5 cm square. Forty-eight of these explants were used in these experiments. The full details of the cultural conditions employed are given elsewhere (Marks *et al.*, 1972). The explants are floated freely onto the surface of Eagle's Medium MEM single strength TC29 (Wellcome Research Labs, Beckenham). In all the experiments described here, pooled normal human serum in a concentration of 10% was added to the culture medium. In eleven cultures, tritiated thymidine was added in a concentration of 1 μCi/ml (specific activity 2 Ci/mmol, batch number 23), during the first 4 h of culture. After exposure during this first 4 h, the explants were washed several times in cold medium and then replaced in a Petri dish containing the same medium supplemented with 10% normal human serum. The culture was then prolonged for a further 44 h. In ten experiments the explants were allowed to proceed in culture for 44 h before addition of tritiated thymidine (1 μCi/ml) to the medium. In addition, seven skin explants were cultured for 4 h only, with medium containing tritiated thymidine in the same concentration as previously. At the end of culture all explants were washed in normal saline and fixed in 10% neutral formalin. After dehydration in graded alcohols they were embedded in paraffin and sectioned at 5 μm. Sections were 'de-paraffinized' and dipped in Ilford nuclear emulsion K2 (Ilford, Nuclear Res. Essex) and exposed at 4° C for periods of 1, 2 and 3 weeks. After exposure they were developed in Kodak developer D19. Before inspection, the autoradiographs were stained with haematoxylin and eosin and mounted in 'Permamount'. In eight cultures colcemid was added for the final 4 h of culture in a concentration of 0·3 μg/ml and at the end of culture the specimens were either frozen or fixed in 10% neutral formalin before histological preparation and sectioning at 5 μm. In addition, twelve explants showing epiboly were sectioned throughout the entire specimen and the serial sections were scanned for mitotic figures.

RESULTS

Autoradiographic

The number of labelled basal and suprabasal cells was expressed as a percentage of the total number of basal cells counted (labelling index). Between 500 and 1000 cells were counted for each specimen.

TABLE 1. Mean labelling indices (±SEMs) for the three cultural conditions described

4-h cultures	48-h culture	
4-h pulse	First 4-h pulse	Last 4-h pulse
8·4±0·8	6·3±1·1	1·6±0·5

The percentages of labelled cells for each of the three cultural conditions described is given in Table 1. The labelling index of explants cultured for 4 h only in the presence of tritiated thymidine differed only slightly from the labelling index of explants cultured for 2 days, but pulsed with thymidine in the first 4 h. In addition, 48-h cultured explants exposed to tritiated thymidine in the last 4 h of culture have a comparatively low labelling index.

A special note was taken of the site of labelled cells in the explants cultured for 48 h. There did not appear to be any special localization of labelled cells in those cultures labelled in the first 4 h and labelled cells certainly did not occur more frequently in the migrating portion or nearby, than elsewhere in the explant (Fig. 1). The migrating portion of epidermis and adjoining edge of explant did not appear to be labelled in specimens pulsed in the last 4 h of a culture. When labelled cells divide, the daughter cells have half the number of autoradiographic grains over them. This produces a population of cells with half the number of autoradiographic grains. No such population was seen in those specimens that had been cultured for 48 h but labelled in the first 4 h.

Mitoses

No normal metaphase figures were seen in those specimens that had been cultured in the presence of colcemid for the last 4 h of a 48-h culture. Only a few abnormal mitotic figures were seen that might have represented abnormal prophase stages in any of the cultured specimens that we have examined.

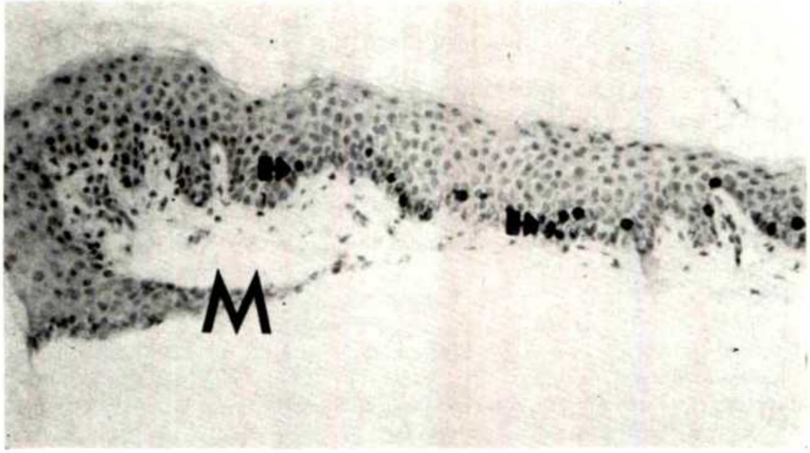

FIGURE 1. Section of human skin maintained in culture for 48 h. A column of migratory epithelial cells can be seen following the free dermal aspect of the specimen (M). A pulse of tritated thymidine was supplied in the first 4 h of culture and labelled cells are indicated by arrows (H & E, × 65).

COMMENT

The fact that skin cultured for 48 h, but labelled for the first 4 h, did not have a higher labelling index than those explants cultured only for 4 h in the presence of tritiated thymidine, suggests that mitosis did not follow DNA synthesis in these cells. In addition, explants that were labelled in the last 4 h of a 48-h culture showed a very low labelling index compared to that of explants labelled in the first 4 h. This may reflect a decreased tissue viability. However, it may also be a reflection of a more specific interference with the delicate mechanism of mitosis as the tissues appear viable and possess a full complement of histochemical reactions (Marks *et al.*, 1972). The lack of mitotic figures, even in the colcemid treated specimens, also suggests that there is some interference with the mechanism of mitosis in culture in normal adult human skin *in vitro*. Hell & Cruickshank (1963) using guinea-pig skin to study wound healing also found that mitotic activity was considerably reduced *in vitro* and did not believe decreased tissue viability was the cause.

Our findings suggest that epithelial migration can take place around the free dermal aspect of cultured explants of skin independently of mitotic activity, i.e. there has been active epidermal movement dissociated from cell division. Both Lachapelle & Gillman (1969), and Hell & Maibach (1972) have shown that the labelling index of skin *in vitro* is similar to that obtained by *in vivo* injection, at least for short periods of time. Thus, as our findings reveal that mitosis does not follow this normal S-phase it seems that when skin is removed a period of mitotic shock ensues. It has been suggested that a similar situation can occur normally in mouse skin (Gelfant, 1969) and this arrested phase of the mitotic cycle, during the G2 period of the cell cycle, has been thought to give rise to a discrete physiological population.

A number of workers have examined adult human skin *in vitro* for mitotic figures and in some cases have succeeded in finding them (Medawar, 1948; Sarkany *et al.*, 1965; Washburn, 1954; Reaven & Cox, 1968). However, we submit that our findings suggest that migration *in vitro* during the first 48 h can take place in the absence of mitosis. In addition, it appears that the trauma of removing and preparing the skin for culture results in some way in interference with entry into the phase of mitotic spindle formation.

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Lichen simplex: morphodynamic correlates*

R.MARKS AND G.C.WELLS

Department of Pathology and Institute of Dermatology,
St John's Hospital for Diseases of the Skin, London WC2H 7BJ

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SUMMARY

The rate of epidermal cell proliferation has been examined by autoradiographic methods in lichen simplex chronicus in sixteen patients. The corrected labelling index was found to be 25·9% in eleven biopsies removed 50 min to 1 h after intracutaneous injection of tritiated thymidine (i.e. similar to that in psoriasis). Twelve biopsies were removed 1–8 days subsequent to injection with tritiated thymidine, and in the autoradiographs that resulted it was found that the rate of travel of labelled cells was not fast as in psoriasis. The possible explanations for the finding of a labelling index similar to that of psoriasis but a slower transit time include; the presence of a greater epidermal volume in lichen simplex; the inclusion of 'high level labelled cells' in the labelling index; and epidermal damage from the trauma of scratching resulting in intra-epidermal cell death of labelled cells.

Lichen simplex, or circumscribed neurodermatitis, is characterized histologically by epidermal hypertrophy which can at times reach massive proportions. The acanthosis and enlargement of epidermal ridges are often more pronounced than in psoriasis, although in contrast to psoriasis areas of hyperkeratosis are interspersed with areas of parakeratotic scaling. Despite these similarities on the one hand, and differences on the other, we can not trace any investigations that have contrasted the epidermal cell kinetic situation with that in psoriasis. In this paper we report our findings in twenty-three patients with lichen simplex in whom we have studied the rate of epidermal cell proliferation by autoradiographic methods in order to understand this type of epidermal reaction pattern.

PATIENTS AND METHODS

Sixteen patients with typical lichen simplex were investigated. There were ten men and six women and their average age was 59 years. The circumscribed lichenified patches on these patients involved the arms, elbows or wrists (twelve) and calves or ankles (four) and had mostly been present for more than 2 years. Twenty-three biopsies were taken from these sixteen patients after intracutaneous injection of the biopsy sites with 10 μCi of tritiated thymidine in 0·1 ml normal saline (specific activity 26 Ci/mmole, Radiochemicals Amersham Batch No. 18). Eleven of these biopsies were removed 50 min to 1 h after injection with thymidine, and twelve were removed at longer intervals of from 1 to 8 days. After removal the biopsies were fixed in 10% neutral formalin and dehydrated in graded alcohols before embedding in paraffin. Sections were cut (5 μm), deparaffinized, taken to water, and dipped in Ilford nuclear emulsion K2. The 'dipped' slides were then exposed for periods of from 2 to 4 weeks at 4° C in the dark, and then developed in Kodak D19 developer. The developed slides were then stained in haematoxylin and eosin.

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* Based on a paper presented at the British Association of Dermatology, July 1972.

Representative sections from each biopsy were photographed in a standard way. From the photomicrographs, the ratio of the length of the basal layer to the length of the top of the granular cell layer (B/G ratio) was calculated for each slide. The mean cell thickness of the epidermis was also calculated by direct measurement at ten random points.

RESULTS

Labelling index

The mean labelling index for the eleven biopsies removed between 50 min and 1 h after intracutaneous injection with tritiated thymidine was $18 \cdot 5 \pm 2 \cdot 8$ (SE mean). After applying the correction factor of $\times 1 \cdot 4$ advocated by Weinstein & Frost (1969) the labelling index becomes 25·9. There was some variation in the degree of labelling within several of the specimens although in general there was a uniform high rate of labelling (Fig. 1). Basal cells appeared to be especially heavily labelled where there was a marked inflammatory reaction in the dermis (Fig. 2). Areas of hyperkeratosis and parakeratosis showed equally heavy labelling (Fig. 3).

In several instances labelled cells were visible within the middle of the thickened epidermis and some of these 'high level labelled cells' were surrounded by a clear space (Fig. 4). These were believed to be Langerhans cells as they were identical in position with ATPase positive dendritic cells seen in specimens prepared from biopsies of lichenified areas (Wells & Marks, 1972).

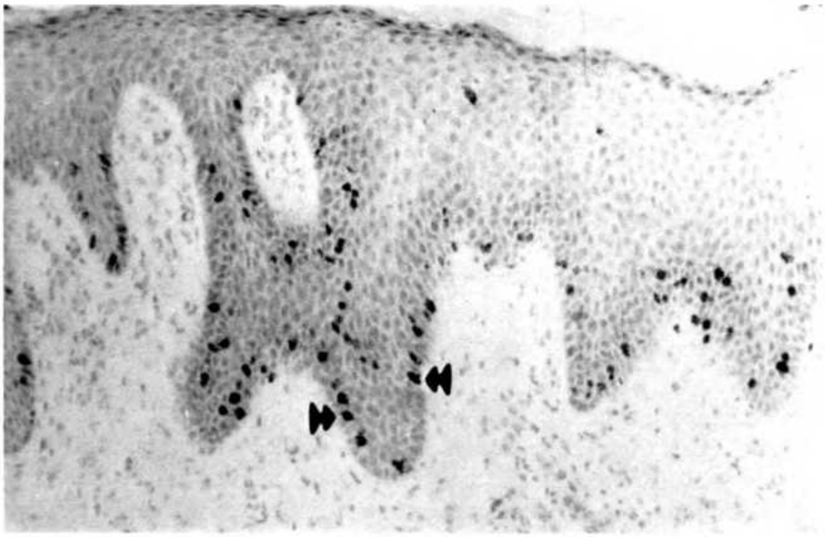

FIGURE 1. Autoradiograph prepared from a patch of lichen simplex after injection of tritiated thymidine 50 min previously. There are a large number of labelled cells (arrowed) in the basal layer (H & E, ×65).

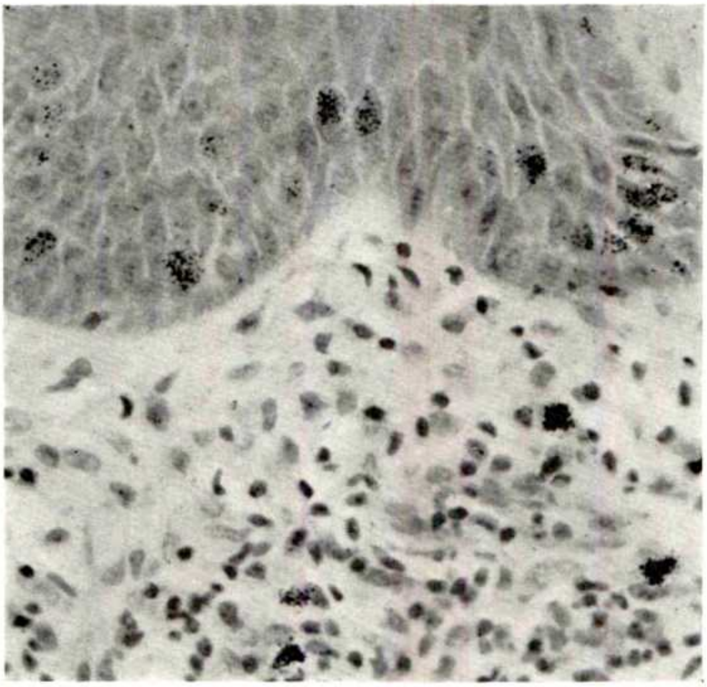

FIGURE 2. Autoradiograph to show a high labelling index over an area of dermal inflammation. Prepared from an area of lichen simplex after injection with tritiated thymidine 50 min earlier (H & E, × 150).

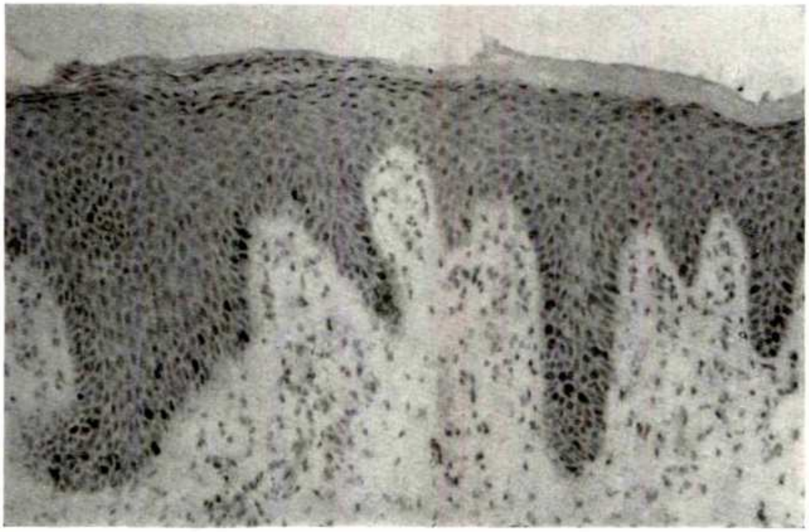

FIGURE 3. In this photomicrograph there is approximately the same degree of labelling of basal cells beneath areas of hyperkeratosis and parakeratosis. From an area of lichen simplex injected with tritiated thymidine 50 min previously (H & E, × 65).

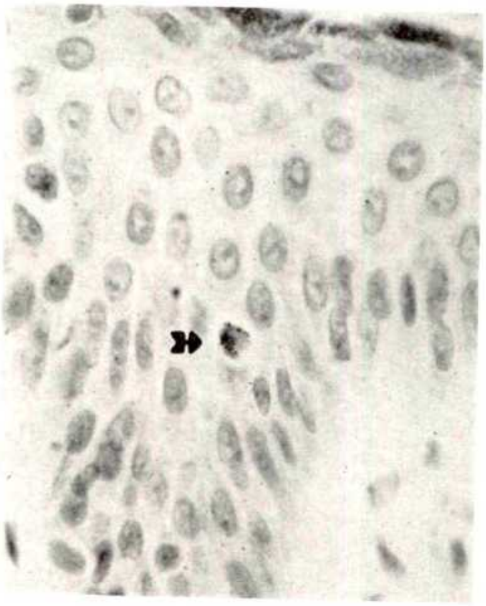

FIGURE 4. A labelled cell can be seen (arrowed) in a clear space within the epidermis. It is probable that this labelled 'clear cell' represents a labelled Langerhans cell (H & E, ×90).

Transit times

The highest point reached within the epidermis by labelled cells (ignoring those labelled cells, mentioned above, surrounded by a clear space) for each 'interval' biopsy is indicated in Fig. 5. The rate of travel within the epidermis seemed slower than might have been expected from a consideration of the high labelling indices and from comparison with similar preparations from psoriatic lesions (Fig. 6). However, labelled cells were identified in the granular cells layer in one specimen biopsied 2 days after intracutaneous injection with tritiated thymidine.

Epidermal measurements

The mean *B*/*G* ratio was 2·4 (±0·13) and the mean epidermal thickness was 14·9 (±0·8). In Figs. 7

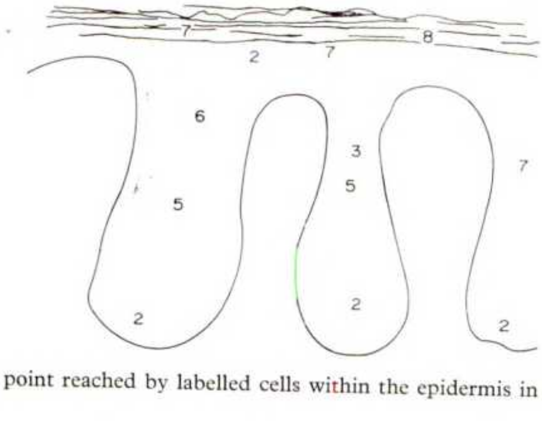

FIGURE 5. Highest point reached by labelled cells within the epidermis in interval biopsies.

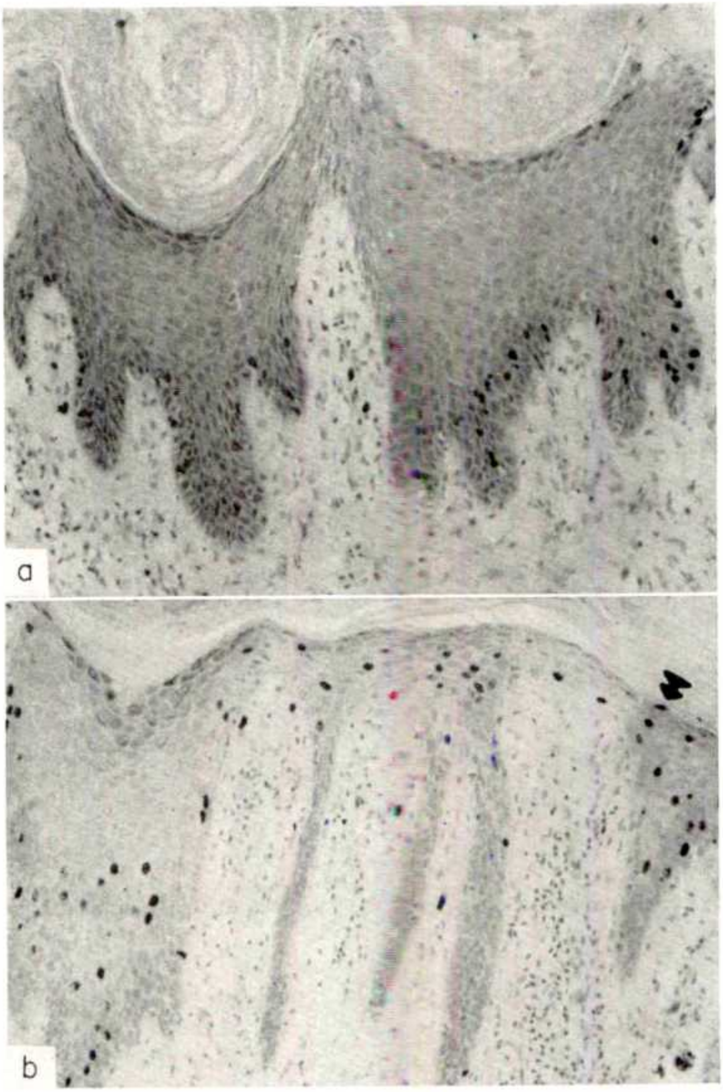

FIGURE 6. (a) Autoradiograph prepared from biopsy taken from a site injected 2 days previously with tritiated thymidine. The labelled cells have not travelled far through the epidermis (H & E, × 65). (b) Labelled cells are scattered through the epidermis in this autoradiograph prepared from a biopsy injected 7 days previously with tritiated thymidine. Despite one labelled cell (arrowed) being seen at the top of the granular cell layer the transit of the labelled cells through the epidermis is relatively slow (H & E, × 65).

and 8 the labelling index has been plotted against the B/G ratio and thickness for each biopsy respectively. The best straight line in both these plots indicates that in general the higher the B/G ratio or epidermal thickness, the higher is the labelling index.

DISCUSSION

The labelling index in psoriasis has been found to be 22·9 (Weinstein & Frost, 1970). Lichen simplex is a very stable condition from the clinical point of view and we were surprised to find that the rate of

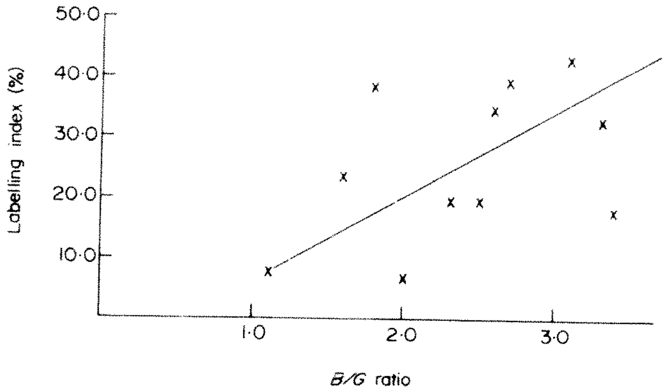

FIGURE 7. The labelling index plotted against the *B/G* ratio for each biopsy. The line drawn is the 'best straight line'.

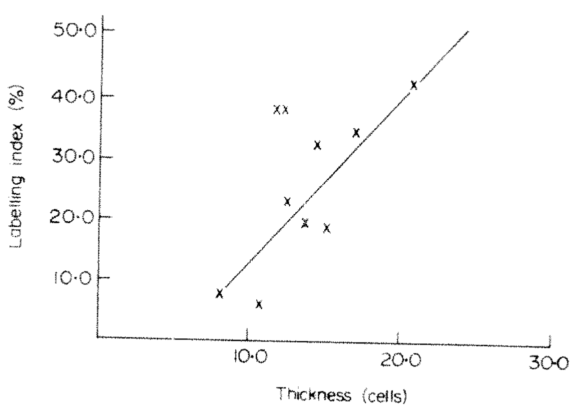

FIGURE 8. The labelling index plotted against the epidermal thickness for each biopsy. The line drawn is the 'best straight line'.

epidermal cell proliferation (as evidenced by a labelling index of 25·9%) was similar to or even greater than in psoriasis. However, the rate of transit of labelled cells through the epidermis was not as fast as in psoriasis and it is interesting to speculate on the reasons for this difference between the two conditions. Halprin (1972) has cautioned against uncritical acceptance of transit time data, and implied that the size of the compartment through which the labelled cells have to travel must be taken into account in kinetic studies. The *B/G* ratio of 2·4:1 that we observed in lichen simplex in the investigation is greater than in *B/G* ratio 2·1:1 that has been found for psoriasis (Marks, 1972 to be published). The mean thickness of 14·9 cells found in lichen simplex is also greater than the 9·0 found in psoriasis. Thus, it appears that epidermal volume is greater in lichen simplex than in psoriasis and one reason for the relatively slow movement of labelled cells within the epidermis when compared to the labelling index must be the very large number of keratinocytes within the thickened epidermis that have to be replaced if an equilibrium is to be maintained. There are other possible contributory causes for the disparity between the high labelling index and the relatively slow transit time when compared to psoriasis. Some of the labelled suprabasal cells were probably Langerhans cells (see above) and would contribute at least partially, to a false impression of raised labelling index. Schellander & Marks (1972) have demonstrated that in reactive epidermal hypertrophy there is an increase in the number of 'high level labelled cells' which may amount to 10% of the number of labelled cells. In addition the rubbing and scratching of patches of lichen simplex probably results in intra-epidermal damage so that some labelled cells may never reach the surface, also resulting in a false impression of slow rate of travel of labelled cells. Further possible causes of the disparity include, extensive repair synthesis of DNA, and a delay by some labelled cells before entering mitosis (a G2 block)—both of which could result in a falsely high estimate of the rate of proliferation from the autoradiographic labelling index. However, these last two theoretical considerations are unlikely to apply to lichen simplex.

The relatively slow rate of transit of labelled cells through the epidermis may partially explain why hyperkeratosis as well as parakeratosis is seen in lichen simplex in contrast to the extensive parakeratosis of psoriasis. However, parakeratosis is not necessarily an accompaniment of high rate of epidermal cell output as can be seen from Fig. 3, and epidermal damage can also result in parakeratotic scaling (Christophers & Braun-Falco, 1970; Marks & Black, 1972).

At times lichen simplex can resemble lichen planus clinically, and the epidermal kinetics of the two disorders also show similarities. The hypertrophy of the upper epidermal zones in lichen planus can be regarded as the result of continuous damage to the generative compartment, with the consequent migration into the involved area of healthy epidermal cells which then re-populate the affected epidermis (Marks, Black & Wilson-Jones, 1972). This damage and repair sequence in lichen planus is accompanied by compensatory epidermal cell production. In lichen simplex the high labelling index that we have observed is probably also the result of epidermal damage, but of a different and more chronic variety, that of continual rubbing and scratching. It is interesting to note that lichen planus most often resembles lichen simplex when the process seems to proceed slowly and the damage to the basal zones is less pronounced.

Goldblum & Piper (1954) found that lichen simplex could be induced experimentally in individuals with pre-existing eczema or pityriasis rubra pilaris. In addition, Naylor (1955) was able to induce lichenification in an individual who had atopic eczema using a constant mechanical stimulus, but not in a normal subject who served as a control. Although self-induced trauma plays an important localizing role in the production of lichen simplex, atopic subjects develop it more readily than others and additional as yet unknown factors must be involved in the pathogenesis. An appreciation of the epidermal cell kinetics of lichen simplex allows a more rational therapeutic approach and is at least one step nearer an understanding of this disorder.

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The effect of local temperature changes on sebum excretion rate and forehead surface lipid composition

M.WILLIAMS, W.J.CUNLIFFE, B.WILLIAMSON, R.A.FORSTER,
J.A.COTTERILL AND J.C.EDWARDS

Department of Dermatology, The General Infirmary, Leeds, St James's (University) Hospital,
Leeds, and Harrogate General Hospital

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SUMMARY

The effect of local change in forehead skin temperature on both surface lipid composition and sebum excretion rate was studied in thirty-six subjects. The sebum excretion rate varied directly with local temperature change so that a change in temperature of 1° C produced a change in the sebum excretion rate of the order of 10%.

Significantly less squalene was detected in surface lipid when forehead temperature was decreased but no other temperature dependent variations in the composition of surface lipid were observed.

It is known that the sebum excretion rate varies directly with local change in forehead temperature (Cunliffe, Burton & Shuster, 1970). We have investigated the effect of local temperature changes on skin surface lipid composition. The sebum excretion rate was determined simultaneously.

METHOD

Forty-six observations were made on thirty-six subjects, thirteen males and twenty-three females; twenty-nine subjects had acne vulgaris and eight of these subjects were receiving antibiotics but no topical therapy for this condition. Five of the older subjects had a previous history of acne. The sebum excretion rate was measured on the forehead using absorbent papers by the modified method of Strauss & Pochi (1961) (Cunliffe & Shuster, 1969). Skin surface lipid composition was determined simultaneously for squalene, wax and sterol esters, free fatty acids and cholesterol by a semiquantitative thin-layer technique (Cotterill *et al.*, 1971a).

Forehead sweating was prevented as far as possible by the application of 5% poldine methanosulphonate in alcoholic solution 30 min before the start of the experiment. One side of the forehead acted as a control remaining at room temperature whilst the temperature was increased or decreased on the experimental side of the forehead by the use of a polythene coil (Fig. 1) placed over the absorbent papers. Hot or cold water was circulated through this coil by a high speed pump from a thermostatically controlled water tank and four subjects could conveniently be studied in series at any one time. Skin surface lipid was not collected until the temperature had equilibrated on the 'coil' side of the forehead. The time for equilibration was usually between 10 and 15 min. After equilibration the forehead skin temperature was recorded at 15 min intervals by a thermocouple reading to ±0·1° C. Surface lipid was collected for 3 h in each subject and analysed without delay to avoid any changes in skin surface lipid composition due to storage (Downing, 1970). All surface lipid collections were made at the same time of day to avoid any circadian variations in either the sebum excretion rate (Burton, Cunliffe & Shuster, 1970) or skin surface lipid composition (Cotterill *et al.*, 1971b).

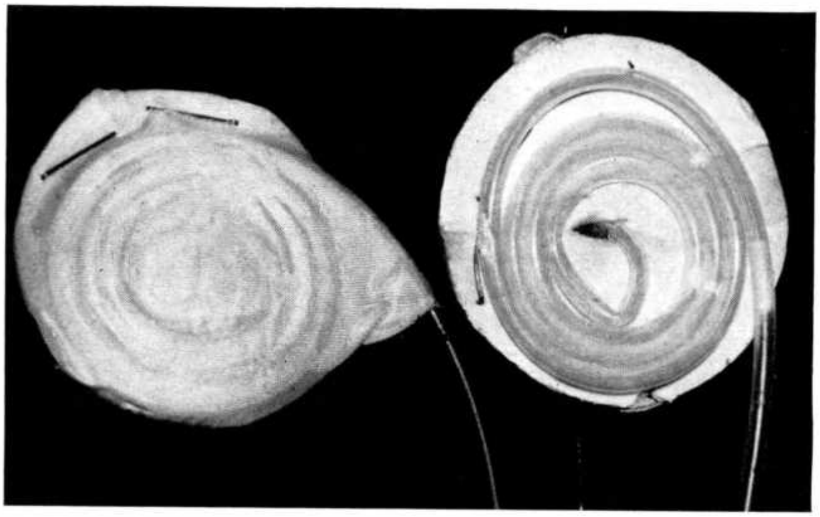

FIGURE 1. The polythene coils used.

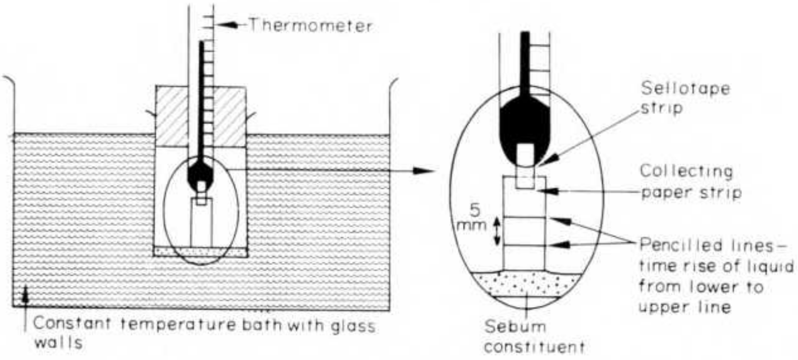
Thermometer
5 mm
Sellotape strip
Collecting paper strip
Pencilled lines– time rise of liquid from lower to upper line
Constant temperature bath with glass walls
Sebum constituent

FIGURE 2. The apparatus used to study the effect of local temperature change *in vitro* on absorption of sebum constituents by absorbent papers.

The effect of temperature change on the absorption of certain sebum constituents by collecting papers was investigated *in vitro* by a modification (Fig. 2) of the method of Cunliffe *et al.* (1970).

RESULTS

The effect of an increase in forehead skin temperature on sebum excretion rate and surface lipid squalene content is shown in Table 1. Temperatures ranging from 38·4 to 39·9° C were achieved on the heated side. The mean increase in temperature was 3·6° C.

The effect of a decrease in forehead skin temperature on sebum excretion rate and surface lipid

TABLE 1. The effect of a local increase in forehead skin temperature ($T°$ C) on sebum excretion rate (SER) and squalene content

	Control side	Heated side	N	$2P$
Mean skin temperature	35·4	39·1	—	—
Means SER (μg/cm^2/min)	1·03	1·46	11	0·001
Mean squalene (%)	13·4	12·9	12	0·5

TABLE 2. The effect of a local decrease in forehead skin temperature ($T°$ C) on sebum excretion rate (SER) and squalene content

		A	B	C	D
Number of subjects		15	19	34	5
Mean skin temp.	Control side	34·9	34·9	34·9	*34·7
	Cooled side	31·4	28·3	29·7	28·9
Mean decrease in skin temperature		3·5	6·6	5·2	5·8
Mean SER (μg/cm^2/min.)	Control side	1·41	1·10	1·24	1·15
	Cooled side	1·34	0·90	1·10	1·17
Probability	($2P$)	0·4	0·001	0·005	0·7
Mean squalene (%)	Control side	15·6	15·6	15·6	15·1
	Cooled side	14·2	14·0	14·1	14·5
Probability	($2P$)	0·2	0·01	0·005	0·4

A, Subjects showing smaller temperature decrease; B, subjects showing larger temperature decrease; C, all subjects; D, subjects whose acne has spontaneously regressed.

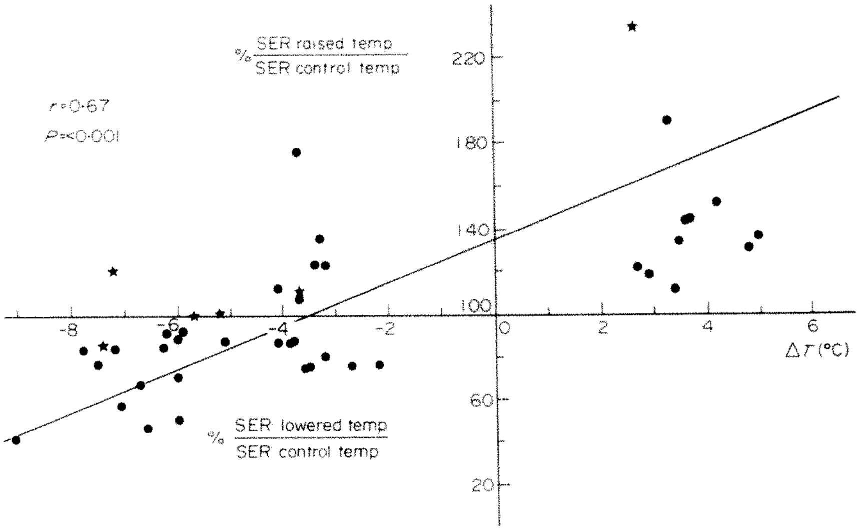

FIGURE 3. The effect of temperature on sebum excretion rate.

squalene content is shown in Table 2. Local temperature on the cooled side varied between 26·1 and 32·9° C and the mean decrease in temperature was 5·2° C.

The effect of local temperature change on sebum excretion rate is shown in Fig. 3. Sebum excretion rate varies directly with local temperature change. The line drawn represents the geometric mean of the regressions of x upon y and of y upon x calculated by the method of least squares. From this line it can be calculated that a local change of temperature of 1° C produces a 10% change in sebum excretion over the temperature range studied.

Two groups of subjects failed to show a significant decrease in sebum excretion rate with a decrease in local temperature (Fig. 3). Thus subjects with a previous history of acne and subjects in whom the local decrease in forehead temperature was less than 4° C failed to show a significant decrease in sebum excretion rate. However, when all observations in all subjects were analysed as a whole the correlation coefficient ($r = 0{\cdot}66$) showed a highly significant relationship between local temperature change and sebum excretion rate ($P < 0{\cdot}001$).

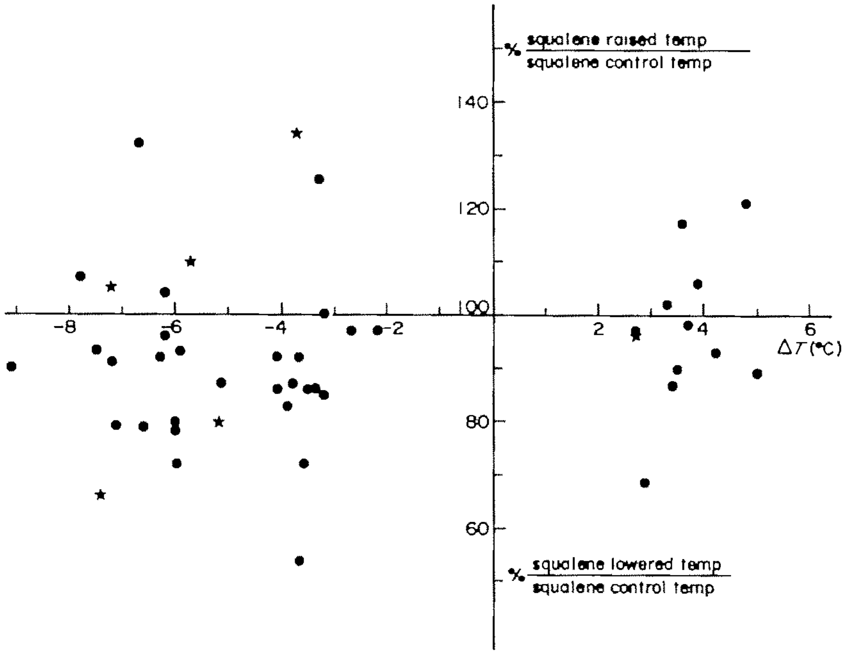

FIGURE 4. The effect of temperature on surface lipid squalene content.

There were no significant changes in the percentages of surface lipid wax and sterol esters, triglycerides, free fatty acids and cholesterol with changes in local temperature. The effect of local temperature change on the percentage of forehead surface lipid squalene is shown in Fig. 4.

Analysis using a paired student '*t*' test showed that a decrease in forehead temperature led to a significant decrease in skin surface lipid squalene ($P < 0{\cdot}005$), although regression analysis showed that this pattern of variation could not be described by a simple straight line. There was no significant increase in the percentage of surface lipid squalene with an increase in temperature. There was no correlation between the change in sebum excretion rate and the percentage of squalene present in the sebum ($r = 0{\cdot}36$).

An *in vitro* study of the temperature dependence of the collecting paper absorption of three sebum

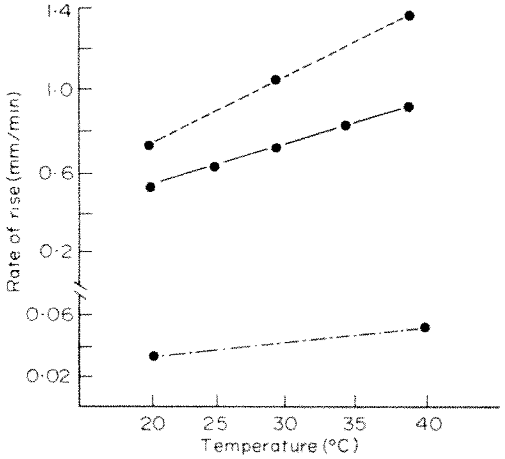

FIGURE 5. The *in vitro* effect of temperature change on the constituents of sebum. – – –, Caprylic acid; ——, squalene; – · –, oleic acid.

components showed (Fig. 5) that considerable differences exist between the rates of absorption of different sebum components.

DISCUSSION

Our results confirm the earlier observation of Cunliffe *et al.* (1970) that the sebum excretion rate varies directly with temperature and that a change of 1° C will produce a change in the sebum excretion rate of the order of 10%.

It is unlikely that our findings can be explained in terms of changes in the rate of sebum production by the sebaceous gland as it is known that the rate of sebaceous gland turnover is of the order of 7 days and therefore is relatively slow (Epstein & Epstein, 1966).

The possible mechanisms involved in the variation of sebum excretion rate with temperature have been discussed by earlier workers (Cunliffe *et al.*, 1970) who also showed that absorption of sebum by collecting papers *in vitro* was temperature dependent. It is known that viscous flow in a tube varies with the fourth power of the radius and so it is possible that changes in sebaceous duct or pore size with temperature may effect the rate of delivery of sebum to the skin surface. However, preliminary evidence suggests that forehead sebaceous pore diameter varies with local change in temperature and hydration (Williams, Cunliffe & Gould, 1972).

It is of interest that not all patients showed a significant reduction in sebum excretion rate when the skin temperature was reduced and a local temperature decrease of more than 4°C was necessary to produce significant decrease in sebum excretion rate. It may be worthwhile to investigate further the sebum excretion rate around this particular temperature difference in an attempt to assess sebum viscosity *in vivo*.

Furthermore, in contrast to patients with acne all five subjects with a previous history of acne showed no significant decrease in sebum excretion during cooling. This could mean that the sebum of patients with acne tends to become more viscous than in patients with a previous history of acne when the skin temperature is reduced. However, acne patients when treated with long term oral tetracycline do not differ in their response to local temperature change from untreated patients. This may suggest that the physico-chemical changes occurring in sebum with cooling differ in patients whose acne has cleared spontaneously when compared to those acne subjects who have responded to oral tetracycline.

It has been claimed that squalene and to a lesser extent free fatty acids may influence the fluidity of surface lipid (Lewis & Hayward, 1971). The lack of correlation between sebum excretion rate and squalene content and the absence of any significant changes in free fatty acid content with temperature change argues against the possibility that temperature mediated changes in sebaceous composition influence the rate of delivery of sebum to the skin surface.

There is, however, no doubt that the surface lipid content of squalene is effected by a local decrease in skin temperature and unless precautions are taken to standardize methods of collecting then errors may creep into interpretation of data.

Some of our present findings could be explained by either changes in the absorbent properties of the papers at different temperatures or temperature mediated viscosity changes. Our *in vitro* work demonstrated a striking relationship between the type of lipid, temperature and absorption. The general rise in absorption rate with temperature may be explained by a fall in viscosity of the respective components.

Finally, it should be stressed that sebum is a substance with complex physico-chemical properties containing a variety of lipids, some of which are solids at body temperature and other such as squalene and oleic acid which may function as solvents.

However, our biochemical techniques are only semiquantitative and more detailed analysis of the sebum constituents by for example, gas liquid chromatography might have demonstrated changes which our less sensitive analytical methods failed to detect.

ACKNOWLEDGMENTS

The authors are grateful to the Wellcome Trust, the Leeds Regional Hospital Board, the Board of Governors of the United Leeds Hospitals Group and the Leeds and West Riding Medical Research Trust and Vick International for financial support. We also thank Mrs J.Davies and Mrs J.R.Middleton for technical assistance and Mrs L.Lane for secretarial help.

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Sebum excretion in Parkinsonism

J.L.BURTON, MARGARET CARTLIDGE, N.E.F.CARTLIDGE*
AND SAM SHUSTER

Wellcome Laboratories for Research into Skin Disease, University Department of Dermatology,
Newcastle-upon-Tyne, and * Department of Neurology, Royal Victoria Infirmary,
Newcastle-upon-Tyne

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SUMMARY

The sebum excretion rate on each side of the forehead was measured in nineteen patients with Parkinsonism, and related to the severity of the neurological disorder on each side of the body. The absence of a significant correlation between sebum excretion and the severity of Parkinsonism on the two sides suggests that the seborrhoea of Parkinsonism is unlikely to be due to a peripheral neurological effect.

The mechanism of the seborrhoea of Parkinsonism is obscure. We previously found that laevodopa reduced the seborrhoea of Parkinsonism and suggested that this effect might be mediated centrally via the pituitary gland (Burton & Shuster, 1970; Burton, Cartlidge & Shuster, 1972), but we could not exclude a neurological mechanism. If this were the case however we should expect the degree of seborrhoea on each side of the forehead to be related to the severity of the neurological disease on each side and we have now examined this possibility.

METHODS

The sebum excretion rate (SER) in ten male and nine female patients with Parkinsonism was measured on each side of the forehead by the method of Strauss & Pochi (1961) as modified by Cunliffe & Shuster (1969). In ten of the patients the measurement was repeated and the mean value was taken as the SER. Every patient was assessed independently by a neurologist who had no knowedge of the SER, using a modification of the Northwestern Disability Scale (Canter, de la Torre & Mier, 1961). The severity of each of five physical findings—postural stability, bradykinesia, finger dexterity, tremor and rigidity was evaluated separately on each side of the body and each sign was graded from 0 (normal) to 4 (severe). The severity of the disease on each side was then expressed on a numerical scale from 0 to 20.

We were unable to study any untreated Parkinsonism patients. Five of the patients had previously undergone a unilateral thalamotomy, and all the patients were receiving oral anticholinergic therapy. None had received L-dopa before the SER was measured.

The SER was also measured in thirty-seven male and forty-eight female control subjects of the same age group. These were patients attending the skin out-patient department with localized dermatoses such as tinea pedis, verrucae, varicose ulcers, etc. and their healthy relatives.

RESULTS

The difference between the mean of the SER measurements from both sides of the forehead of each patient and the mean control value for the corresponding age and sex, is shown in Fig. 1. The mean SER for the patients with Parkinsonism was increased compared with the controls in the male patients by $0{\cdot}19 \pm$ SE $0{\cdot}12$ $\mu g/cm^2/min$ and in the females by $0{\cdot}23 \pm$ SE $0{\cdot}13$ $\mu g/cm^2/min$, but neither of these increases was significant ($P > 0{\cdot}05$). When the data from males and females was pooled however the increase of $0{\cdot}21 \pm$ SE $0{\cdot}09$ $\mu g/cm^2/min$ was just significant ($P < 0{\cdot}05$).

The severity of the Parkinsonism and corresponding SER for each patient is plotted in Fig. 2. There was no significant correlation for male patients ($r = 0{\cdot}19$) but for the females the correlation was just significant ($r = 0{\cdot}65$, $P < 0{\cdot}05$).

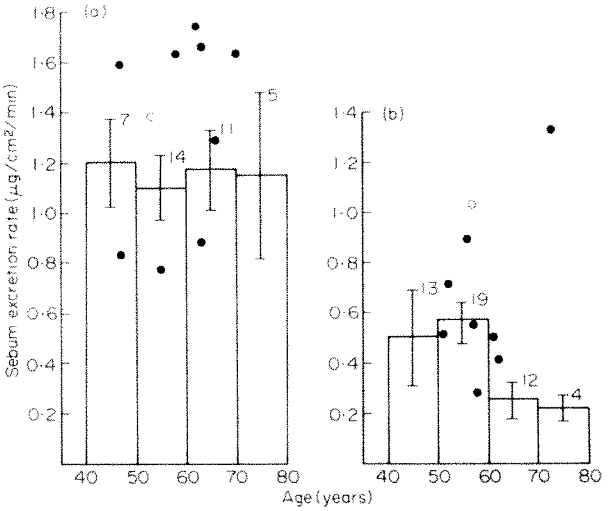

FIGURE 1. (a) Males, (b) females. Vertical bars, mean SE (controls); ●, idiopathic Parkinsonism; ○, post-encephalitic Parkinsonism.

To assess whether the SER was greater on the more severely affected side in each patient we plotted the difference in SER on the two sides of the forehead against the difference in severity of the Parkinsonism on each side of the body (Fig. 3). There was no significant correlation between these differences ($r = 0{\cdot}13$, $P > 0{\cdot}05$).

DISCUSSION

The many clinical observations of seborrhoea in Parkinsonism (Cohn, 1920, Krestin, 1927) have been confirmed quantitatively by Serrati (1938), Kvorning (1952) and Pochi, Strauss & Mescon (1962) and the present study also showed an increased mean SER. It is clear however both from these authors and the present findings that not all patients with Parkinsonism have seborrhoea.

The pathological lesion in Parkinsonism is variable and it seems likely that the precise site of the lesion may be important in causing seborrhoea. It is also possible that the SER may have been reduced

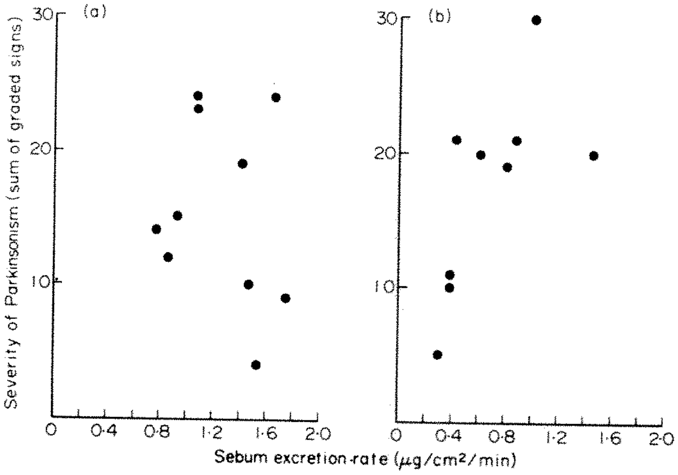

FIGURE 2. (a) Males, $r = 0{\cdot}19$, $P > 0{\cdot}05$. (b) Females, $r = 0{\cdot}65$, $P < 0{\cdot}05$.

in our patients by their anticholinergic treatment. A peripheral effect is unlikely, because we have shown that prolonged topical application of an anticholinergic agent has little effect on the SER (Cartlidge, Burton & Shuster, 1972), but a central effect has not been excluded.

Seborrhoea correlated better with the overall severity of the disease in the female than in the male. This may be because fewer of the males had seborrhoea, but more studies will be required to confirm this.

Our present observations do however show that there is no significant correlation between SER and severity of Parkinsonism on the two sides of the body. This suggests that the seborrhoea of Parkinsonism is not due to a neurological effect. On the other hand, evidence to support an endocrine mechanism is accumulating and we have recently suggested that the seborrhoea of Parkinsonism is due to a decreased hypothalamic inhibitory effect on a pituitary sebotrophic hormone. We believe this

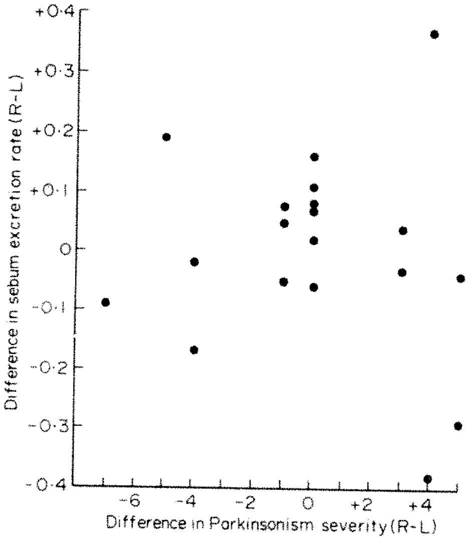

FIGURE 3. Males and females pooled, $r = 0{\cdot}13$, $P > 0{\cdot}05$.

explains why L-dopa decreases sebum secretion in Parkinsonism only when there is seborrhoea (Burton *et al.*, 1972).

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Some effects of fats with dietary carbohydrates on the lipids on the surface of the skin

IAN MACDONALD

Department of Physiology, Guy's Hospital Medical School, London SE1 9RT

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SUMMARY

Young men and women were given diets containing various carbohydrate mixtures given with either sunflower seed oil or cream and the triglyceride and cholesterol concentration on the surface of the skin above the right scapula was determined. In men, the triglyceride level on the surface of the skin was reduced by sunflower seed oil. In young women the nature of the dietary constituents seemed to have no marked effect on the lipids on the surface of the skin.

The skin, like any other tissue, is affected by the nature of the food eaten and it has long been recognized that certain dietary constituents such as vitamins, may have a special role in the metabolism of skin. The part played by dietary carbohydrates and fats in skin biochemistry is less well defined and although it was taught some 40 years ago that dietary carbohydrates should be restricted in the treatment of seborrhoeic states (Barber, 1930) there is still little information about this aspect of the skin. It was therefore decided to study the composition of the lipid on the surface of the skin while the type of fat and the type of carbohydrate in an experimental diet were altered; the proportions of fat, carbohydrate and protein in the diet remaining unchanged.

MATERIALS AND METHOD

Ten healthy male students aged 18–21 years were given in liquid form a diet composed, by weight, of 60% carbohydrate, 27% fat and 13% protein as calcium caseinate; these proportions being similar to those found in Western diets. The intake of the diet was approximately 40 Cal/kg body weight/day and was adjusted to keep the body weight constant. A vitamin capsule (BPC) was taken by each subject on each day of the diet which lasted 5 days. There were at least 10 days free-choice diet between successive experimental periods.

As the fasting plasma triglyceride level of men and premenopausal women responds differently to dietary carbohydrates (Macdonald, 1965) it was decided to give seven young women aged 18–21 years diets similar to those given to the men.

The fats used were sunflower seed oil or 'double' cream [48% volume = fat]. These fats were chosen because of the high proportion of polyunsaturated fatty acids in sunflower seed oil and the very low level of these fatty acids in cream. The carbohydrate mixtures were as follows: (a) 2/5 fructose + 3/5 raw corn starch (b) 2/5 fructose + 3/5 glucose (c) 2/5 glucose + 3/5 raw corn starch.

These mixtures were chosen because it is not possible to give more fructose without the risk of causing osmotic diarrhoea.

Samples of skin surface lipid were obtained after a 12 hr fast on the morning the diet started and on the penultimate and final mornings before the subject resumed his free-choice diet.

Samples of skin surface lipid were obtained using a modification (Llewellyn, 1968) of the cup method (Broughton *et al.*, 1955). The lipid solvent was filtered through fat-free shark skin filter paper and then evaporated to dryness. The lipid was re-dissolved and the cholesterol concentration measured on an aliquot by the method of Block, Jarrett & Levine (1966). The triglyceride concentration was determined by an automated method based on that described by Lofland (1964). All the estimations of triglyceride and of cholesterol of a subject on a particular diet were done on the same occasion.

RESULTS

The mean amount of triglyceride and cholesterol on the surface of the skin above the right scapula (expressed as $\mu g/cm^2$) before and on day 4 and 5 of each diet are seen in Tables 1 and 2. There is no obvious explanation for the wide variation found in pre-diet values though the large standard errors seen in the women could be due to fluctuations in skin surface triglyceride and cholesterol associated with the menstrual cycle (Macdonald & Clarke, 1970).

TABLE 1. The mean (and SE) of the amount of triglyceride and cholesterol ($\mu g/cm^2$) in *men* before and after 4 and 5 days on diet

Diet		Day	Triglyceride			Cholesterol		
			0	4	5	0	4	5
Sunflower:	$\bar{x}$		40·4	34·0	33·5	3·9	3·1	2·7
Fructose:starch	SE ±		3·70	2·88	2·78	0·36	0·18	0·13
Cream:	$\bar{x}$		31·2	33·1	33·1	3·1	3·4	3·0
Fructose:starch	SE ±		6·49	5·41	4·96	0·23	0·32	0·31
Sunflower	$\bar{x}$		35·7	30·1	26·9	4·1	3·2	2·6
Fructose:glucose	SE ±		4·06	3·00	2·85	0·63	0·43	0·19
Cream:	x		38·5	39·8	40·1	2·9	2·9	2·8
Fructose:glucose	SE ±		3·59	3·58	3·46	0·27	0·31	0·23
Sunflower:	$\bar{x}$		25·8	21·1	19·6	5·3	4·1	3·8
Glucose:starch	SE ±		2·06	1·86	2·16	0·61	0·67	0·54
Cream:	$\bar{x}$		53·3	52·4	53·7	2·6	2·5	2·6
Glucose:starch	SE ±		7·49	6·63	6·43	0·32	0·30	0·34

In view of the wide between-individual variation the pre-diet value for each subject was subtracted from his or her comparable values on days 4 and 5. Lines were then fitted by the method of least squares to all these values for each diet and the slopes and their standard error are seen in Tables 3 and 4.

On all diets containing sunflower seed oil, irrespective of the carbohydrate mixture accompanying the oil, the men had a significant fall in both triglyceride and cholesterol on the surface of the skin. In the women, no significant change in the skin surface triglyceride and cholesterol on any of the dietary mixtures was found.

TABLE 2. The mean (and SE) of the amount of triglyceride and cholesterol [$\mu g/cm^2$] in *women* before and after 4 and 5 days on diet

		Triglyceride			Cholesterol		
Diet	Day	0	4	5	0	4	5
Sunflower:	$\bar{x}$	65·6	53·8	51·5	5·4	4·9	4·7
Fructose: starch	SE ±	7·18	5·74	4·11	0·44	0·43	0·21
Cream:	$\bar{x}$	52·1	53·0	45·8	5·3	5·3	4·3
Fructose: starch	SE ±	10·37	12·61	8·84	0·53	0·82	0·48
Sunflower:	$\bar{x}$	57·5	46·5	53·4	5·5	4·6	4·9
Fructose: glucose	SE ±	10·61	7·46	7·24	0·67	0·48	0·52
Cream	x	37·4	38·2	43·6	4·8	5·1	5·1
Fructose: glucose	SE ±	4·87	7·32	8·61	0·49	0·46	0·79
Sunflower:	$\bar{x}$	51·1	58·4	58·3	3·2	3·5	3·1
Glucose: starch	SE ±	10·30	6·02	6·93	0·54	0·42	0·35
Cream:	$\bar{x}$	50·2	58·6	55·8	4·2	4·4	4·7
Glucose: starch	SE ±	10·51	13·40	9·66	0·44	0·56	0·43

TABLE 3. The slope of the line (and SE) of change [$\mu g/cm^2$] in triglyceride and cholesterol against time (days) in *men* on the experimental diets

	Triglyceride			Cholesterol	
Diet	*n*	*b*	SE ±	*b*	SE ±
Sunflower: Fructose: starch	30	−1·4‡	0·39	−0·2†	0·07
Cream: Fructose: starch	30	+0·4	0·46	0	0·04
Sunflower: Fructose: glucose	30	−1·2*	0·54	−0·3†	0·10
Cream: Fructose: glucose	30	+0·3	0·24	0	0·05
Sunflower: Glucose: starch	30	−1·2‡	0·27	−0·3‡	0·08
Cream: Glucose: starch	30	0	0·88	0	0·07

*, $P<0·05$; †, $P<0·01$; ‡, $P<0·001$.

TABLE 4. The slope of the line [and SE] of change [μg/cm^2] in triglyceride and cholesterol against time (days) in *women* on experimental diets

	Triglyceride			Cholesterol	
	n	*b*	SE ±	*b*	SE ±
Sunflower: Fructose: starch	21	−1·5	1·03	−0·1	0·11
Cream: Fructose: starch	18	−0·5	1·13	−0·1	0·11
Sunflower: Fructose: glucose	18	−1·7	1·02	−0·1	0·06
Cream: Fructose: glucose	18	+1·3	1·81	−0·1	0·14
Sunflower: Glucose: starch	21	+0·3	2·31	+0·1	0·05
Cream: Glucose: starch	21	+1·6	1·18	+0·1	0·09

DISCUSSION

The sunflower seed oil in the experimental diets had a consistent effect on the skin surface lipids in the men, both triglyceride and cholesterol levels falling by 20–25% after 5 days on the diet, irrespective of the carbohydrate mixture accompanying the oil. The fall in the triglyceride and cholesterol level on the skin surface could be a reflection of comparable changes that occurred in the serum of the subjects. To test whether there was a relationship between these two pools and as the skin and serum samples were taken within a few minutes of each other, the change in the serum lipid level associated with the diet was plotted against the change in the corresponding skin surface lipid value. No significant correlation was found for either lipid nor was there any significant relation when the percentage fall in a serum lipid was plotted against the percentage fall, in the same subject, of the comparable skin surface lipid. These findings suggest that the influence is not directly via its effect on the concentration of the corresponding lipids in the serum.

It is perhaps not surprising that the lipids on the surface of the skin are not a direct reflection of the corresponding lipids in the serum, as the major contribution to the lipid on the surface of the skin is the sebaceous gland. There are several hypothetical ways in which the nature of the dietary fat in conjunction with the sex of the consumer could affect sebaceous gland output, but the absence of supporting evidence makes each hypothesis equally valid. The study reported here is merely fact-finding and currently there is no likely explanation for the findings even though they may be of clinical interest.

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Neutron activation analysis of trace elements in skin

VI. MANGANESE IN PSORIASIS, VITILIGO AND OTHER DERMATOSES

MOHAMED M. MOLOKHIA AND BENJAMIN PORTNOY

Manchester and Salford Hospital for Skin Diseases, Quay Street, Manchester M3 3HL

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SUMMARY

Manganese levels were estimated in skin biopsies and serum in twenty-one cases of psoriasis and fifteen cases of vitiligo. There was a significant reduction in tissue manganese concentrations in psoriasis compared with normal, while vitiliginous skin contained higher manganese levels than apparently normal surrounding skin. Analysis of serum in both conditions revealed no variations in the concentrations of this element.

There was no association between manganese content and the degree of melanization in seborrhoeic warts, whereas consistently high levels were found in pigmented moles.

Manganese is an essential trace element with specific roles in carbohydrate and lipid metabolism (Underwood, 1971). The concentrations of this element in the epidermis were found to be among the highest in the body (Molokhia & Portnoy, 1969). Manganese levels were also found to vary from one region of skin to another, with the foreskin showing the highest and the plantar skin the lowest (Molokhia & Portnoy, 1970a).

Like other essential trace elements in the body, manganese is associated with a number of enzyme systems through which it can influence metabolic reactions. However, no such systems had yet been identified in the skin apart from arginase, the activity of which was found to increase in the presence of manganese ions (Roberts & Frankel, 1949). The possible association of manganese with the main two enzyme-dependent processes in skin, namely keratinization and pigment formation, has been reviewed previously (Molokhia & Portnoy, 1969).

Analytical data for manganese levels in skin diseases remain very scanty. Using spectrographic analysis, Pyatikop & Artamonova (1968) found a general reduction of managese levels in skin in a number of patients with severe dermatoses. The advantage of combining tissue and blood analysis in such instances would be two-fold. Firstly, it would make it possible to detect any abnormalities in the concentrations of this trace element which could be of a diagnostic value. Secondly, it might reveal any correlation between manganese levels and a particular skin phenomenon, and thus further our understanding of the function(s) of this element in the body.

In the present study, samples representing abnormalities in keratinization and/or pigmentation of skin were analysed for their manganese content. Neutron activation techniques were used in view of their unequalled sensitivity for the detection of this element.

MATERIALS AND METHODS

Interscapular skin was obtained by incision biopsy from eighteen patients with extensive psoriasis (ten females and eight males), ten patients with vitiligo (one female and nine males) as well as from eleven volunteers (two females and nine males) who acted as a control group. Further samples were obtained from six racially dark individuals (all males). In cases of psoriasis and vitiligo, biopsies were taken from the edge of lesions so as to include involved as well as uninvolved skin. Psoriatic scales were collected from neighbouring lesions in ten patients. Mid-morning blood specimens were collected from a psoriatic group of twenty-one, a group of fifteen vitiligo patients and also from a control group of twenty-two, as described previously (Molokhia & Portnoy, 1970b). Other skin lesions analysed included seborrhoeic warts (fifty-four), virus warts (thirty-one), moles (six), basal cell carcinomata (five) and one specimen each from eczema, senile keratosis and cutaneous horn. Three patients with multiple seborrhoeic warts had lesions which showed a variation in colour and specimens were obtained from both light and dark material in the same individual.

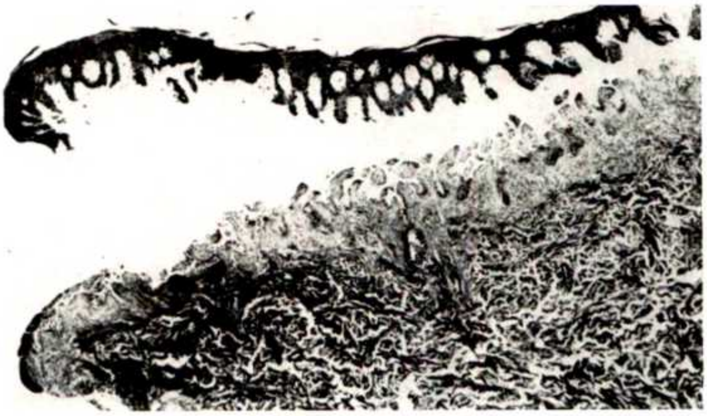

FIGURE 1. Separation of epidermis and dermis in case of psoriasis.

Separation of psoriatic skin into epidermis and dermis was effected satisfactorily by a modified heat method (Fig. 1). The dried specimens were irradiated together with a manganese standard inside a Pye AFM reactor for 2–3 h at a thermal neutron flux of $3{\cdot}6 \times 10^{12}$ n cm^{-2} sec^{-1}. Manganese activity in the samples was separated and counted in a multi-channel pulse height analyser (Molokhia & Portnoy, 1969). All material used in the collection or preparation of samples prior to irradiation was checked for their manganese content and found to contain insignificant amounts.

RESULTS

(a) *Psoriasis*

Manganese levels in psoriatic skin and scales are given in Table 1, together with those found in control skin. Epidermis from both control and psoriatic skin shows higher manganese levels compared with dermis (3–4 times). The figures for involved and uninvolved psoriatic skin are significantly reduced in comparison with normal ($0{\cdot}01 < P < 0{\cdot}05$). Psoriatic scales show levels midway between those in the epidermis and in the dermis.

The results of manganese analysis in serum are shown in Table 2. The psoriatic group shows wider

TABLE 1. Skin manganese levels in psoriasis, vitiligo and pigmented moles

Material	No. of samples	Mn concentration (μg/g dry weight) Epidermis	Dermis
Control skin (white)	11	1·06 ± 0·32	0·28 ± 0·10
Psoriatic skin			
Uninvolved	18	0·45 ± 0·17	0·17 ± 0·09
Involved	17	0·46 ± 0·17	0·16 ± 0·09
Scales	10	0·30 ± 0·16	
Vitiligo			
Lesion	10	1·20 ± 0·42	0·37 ± 0·14
Surrounding skin	10	0·88 ± 0·34	0·23 ± 0·10
Racially dark skin	6	1·02 ± 0·35	0·21 ± 0·09
Moles	6	2·16 ± 0·83	0·47 ± 0·18

range of values compared with the control one and the mean concentration of manganese in psoriatic serum is not significantly different from normal.

(b) *Vitiligo and moles*

The results of manganese analysis in both the vitiliginous and apparently normal surrounding skin are shown in Table 1. There is an increase in manganese levels in both epidermis and dermis of the lesion compared with the surrounding skin and this increase shows a probable significance ($0{\cdot}01 < P < 0{\cdot}05$) in the case of dermis. The values for control skin lie midway between those of the lesion and the surrounding skin. The levels of manganese in serum from a group of fifteen vitiligo patients, shown in Table 2, are not significantly different from normal.

Manganese levels in epidermis and dermis from racially dark skin are close to those from white skin. On the other hand, dermis and epidermis from pigmented moles show highly a significant increase in manganese compared with normal skin ($P < 0{\cdot}001$).

(c) *Seborrhoeic warts*

Manganese concentrations in seborrhoeic warts shown in Fig. 2 have a mean value of 0·60 μg/g dry weight (SD ± 0·39). Here, manganese levels are not related to the duration of the warts or to the age or sex of the patient. The association between manganese content and the degree of melanization could not be definitely ascertained without quantitative melanin estimation. However, high manganese levels seem to be equally shared between dark and light specimens. A comparison between multiple

TABLE 2. Manganese levels in the serum of psoriatic and vitiligo patients compared with normal (μg/g dry weight)

Material	No. of samples	Maximum	Minimum	Median	Mean	± SD
Control	28	0·035	0·010	0·019	0·020	0·005
Psoriasis	21	0·064	0·006	0·021	0·022	0·008
Vitiligo	15	0·045	0·009	0·018	0·018	0·006

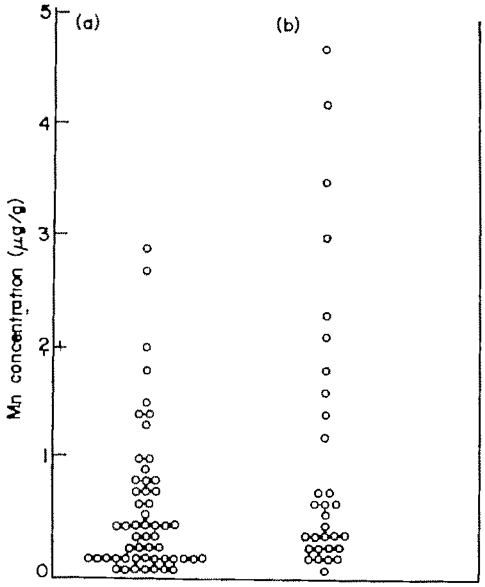

FIGURE 2. Manganese levels in seborrhoeic (a) and common warts (b).

TABLE 3. Variations in manganese levels with pigmentation in patients with multiple seborrhoeic warts

Sex	Age	Site of lesion	Mn μg/g dry weight	
			Dark	Light
♀	69	Upper arm	0·80	1·49
♂	51	Abdomen	0·26	0·54
♀	46	Back	0·26	0·54

TABLE 4. Variations in manganese levels with dry-matter content of seborrhoeic warts

Type of lesion	No. of samples	Mn concentration (μg/g)	
		Mean	SD
With less than one-third dry matter per weight	10	0·95	0·21
With more than two-thirds dry matter per weight	9	0·20	0·07

lesions showing a macroscopic as well as a microscopic variation in pigmentation is given in Table 3. In all cases, light lesions were found to contain higher manganese levels compared with neighbouring dark ones.

Some seborrhoeic warts with the lowest manganese content were found to be composed mainly of horny matter and in these cases the dry : wet weight ratio was highest. Since all specimens were weighed before and after drying, it was possible to relate manganese levels to the dry matter content of the samples. Table 4 shows a comparison between lesions with the greatest dry matter content and those with the lowest. The difference between the two groups is highly significant ($P < 0{\cdot}001$).

(d) *Other dermatoses*

The distribution of manganese levels in thirty-one virus warts is shown in Fig. 2. Their mean value is 1·20 μg/g (SD ± 0·91). Table 5 shows the manganese levels found in a number of lesions from various skin diseases.

TABLE 5. Manganese levels in other dermatoses

Lesion	Site	Mn concentration (μg/g)	
		Epidermis	Dermis
Basal cell carcinoma	Arm	0·40	
Basal cell carcinoma	Face	0·24	
Basal cell carcinoma	Face	0·30	
Basal cell carcinoma	Face	0·45	
Basal cell carcinoma	Face	0·57	
Cutaneous horn	Neck	0·23	
Eczema	Back	0·73	0·17
Senile keratosis	Forearm	0·74	

DISCUSSION

Although in the skin no definite role has as yet been assigned to manganese, there are indications that this essential trace element is implicated in some vital processes there. In the present study, manganese levels were found to be significantly diminished in psoriatic epidermis and dermis. This is in contrast with tissue levels of copper which remain unchanged and those of zinc which are actually increased in psoriasis (Molokhia & Portnoy, 1970b).

However, this 'deficiency', which is unrelated to the severity or duration of lesions, does not seem to be specific for psoriasis and could rather be due to the increased turnover rate of psoriatic epidermis. This may also account for the low manganese content found in basal cell carcinomata. On the other hand, the mean concentration of manganese in psoriatic sera showed no deviation from normal. The mean serum manganese level in the control group is equivalent to 1·76 μg/l, correcting for a mean dry : wet weight ratio of 9·0% and a specific gravity of 1·027. This figure is comparable with the 1·42 μg/l found in normal serum by Cotzias *et al.* (1968).

The common wart, which is another proliferative condition of the epidermis, shows in some cases manganese levels which are much higher than those of normal epidermis. This finding might be related to the increased activity of arginase, which requires manganese as a co-factor, in such lesions (Van Scott, 1951). Arginase is thought to play an important role in keratinization of the epidermis and the internal root sheath (Crounse & Rothberg, 1960). The low manganese content in the horny matter of seborrhoeic warts and in psoriatic scales may indicate that this element is being actively retained by the epidermis together with other vital components of epidermal cells.

The question of manganese content in melanin remains uncertain. Cotzias, Papavasiliou & Miller (1964) reported the presence of high manganese levels in pigmented structures from different animal species and suggested that manganese could be present in very high concentrations in melanin. Our results from human epidermis and epidermal structures do not support this view. Manganese levels in racially dark skin were comparable to those in white skin and vitiliginous structures almost devoid of melanin were found to contain more manganese than the pigmented surrounding skin. At the same time an inverse relationship between the degree of melanization and manganese concentrations was found in three cases of multiple seborrhoeic warts. This last finding would suggest that manganese may be associated with melanin degradation in these lesions.

On the other hand, pigmented moles were found to contain very high manganese levels in both their epidermal and dermal components. However, it could be argued that such moles may possess a proliferating melanocytic population and that this introduces a new factor which may alter the trace element picture. It is interesting to note that in the foreskin, where dendritic cells are present in their highest numbers, manganese levels are also highest (Molokhia & Portnoy, 1970a).

More investigations into the nature of manganese and its compounds in skin are needed. A quantitative study of melanocytes and other dendritic cells in the epidermis of pigmented moles may reveal the relationship between these cells and manganese.

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Pharmacology and Treatment

Dermographism: reduction in weal size by chlorpheniramine and hydroxyzine

CYNTHIA N.A.MATTHEWS, JOHN D.KIRBY, JEAN JAMES, AND ROBERT P.WARIN

Bristol General Hospital, Bristol

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SUMMARY

Regular measurements of the weal size produced by known trauma have been carried out in a series of patients with symptomatic dermographism. The degree of reduction of the weal has been compared after 2 weeks' treatment with chlorpheniramine and hydroxyzine pamoate administered in a double blind manner to thirty-three patients. The doses given were those in common usage and usually well tolerated by patients, namely chlorpheniramine 4 mg three times daily and hydroxyzine pamoate 25 mg three times daily. Hydroxyzine gave a statistically greater reduction of weal size and was also clinically preferred. Hydroxyzine is a weak antihistamine and its mode of action on weal production is unknown, but its clinical efficacy is not likely to be due to its sedative effects.

Statistical proof is given to the observation that weals on exposed sites are smaller than those provoked in areas of skin normally covered.

A weal of more than 2 mm across after frictional trauma with a stylus applied at a pressure of 4900 g/cm^2 occurs in 4·25% of otherwise normal subjects (Kirby *et al.*, 1971). In many subjects this is only a curiosity or at the most a minor nuisance, but in some, considerable irritation is present (symptomatic dermographism). The nature of such dermographism is not fully understood, but it has been shown that in these severe cases passive transfer tests are usually positive (Lorincz, 1956; Johnston & Cazort, 1959) and the substance in the serum responsible for this effect is probably an immunoglobulin (Aoyama, Katsumata & Ozawa, 1970).

It has generally been accepted that oral antihistamine therapy reduces weal size in dermographism and gives some relief in the management of these cases. Hydroxyzine was introduced as a tranquillizer in the form of hydroxyzine hydrochloride ('Atarax' Pfizer) and hydroxyzine pamoate ('Equipose' Pfizer), but it also has an antihistamine and antiserotonin action. It has been used in various patterns of urticaria and it has been observed to give considerable relief (Feinberg *et al.*, 1953; Robinson, Robinson & Strahan, 1956; Seneca, 1957; Eisenberg, 1959; Grater, 1960; Baughman & Jillson, 1963). Its value in physical urticarias has been stressed, and in cholinergic urticaria it was shown to be more effective than a commonly used antihistamine (Moore-Robinson & Warin, 1968). Its effect on induced histamine weals in man has been studied by Dundas, Toogood & Wanklin (1961). Administered over a 24 h period, 75 mg of hydroxyzine hydrochloride was shown to have a greater action than 24 mg chlorpheniramine maleate and 150 mg chlorpromazine. The pharmacological explanation for the action of hydroxyzine is not known, but it has been shown that it is unlikely to be due to its sedative effect (Feinberg, 1953).

It is now possible to measure the weal size in response to different pressure and on different sites of the body, and therefore compare the reduction of the weal size by different substances (James & Warin, 1969).

It was therefore decided to compare the effect of chlorpheniramine, hydroxyzine and a sedative, namely Diazepam, on the degree of itching and the weal size in a group of patients with dermographism.

METHOD

The subjects collected for the trial all presented as routine dermatological out-patients because of their dermographism. They were all included in the trial whether or not they had had previous treatment with antihistamines. They were all tested using the dermographic stylus applied at a pressure of 3650 g/cm^2 and 7400 g/cm^2 on both the upper back and the front of the forearms. The weal sizes were measured directly as described by James & Warin (1969). The subjects were then asked to take three courses of capsules, each lasting 2 weeks, and they were interviewed and retested at the completion of each course, but whilst still taking the tablets. The trial was performed in a double blind manner, neither the patients nor the staff involved knowing in which order the treatment had been used. The drugs were made up in identical capsules and were: hydroxyzine pamoate—25 mg, chlorpheniramine maleate—4 mg, Diazepam—2 mg. One capsule of one of the drugs was given three times a day.

TABLE I

Drug	Site	Load (g/cm^2)	Mean within subject reduction of weal size (control treated) (mm)	*P**
Hydroxyzine	Arm	3650	+1·14	0·005
Hydroxyzine	Arm	7400	+1·67	0·001
Hydroxyzine	Back	3650	+1·25	0·001
Hydroxyaine	Back	7400	+2·17	0·001
Chlorpheniramine	Arm	3650	+0·69	0·01
Chlorpheniramine	Arm	7400	+1·00	0·005
Chlorpheniramine	Back	3650	+0·87	0·001
Chlorpheniramine	Back	7400	+1·29	0·001

**P*, Probability that observed reductions could have arisen by chance from Student's '*t*' test.

Forty-five patients commenced the trial, but six of the early subjects refused to continue, complaining that their dermographism had become much worse. It was found in each case that this occurred during the course of Diazepam and this therefore was eliminated from the trial and only hydroxyzine and chlorpheniramine were used. Six other subjects failed to complete the trial, one because all symptoms had cleared and the others for unknown reasons failed to attend. Thirty-three subjects were therefore available for the comparisons.

TABLE 2

Site	Load (g/cm²)	Mean within subject difference in weal size (hydroxyzine–chlorpheniramine) (mm)	P
Arm	3650	−0·50	0·05
Arm	7400	−0·82	0·025
Back	3650	−0·47	0·3
Back	7400	−0·85	0·025

RESULTS

Analysis of the figures gave the results shown in Table 1 which show that both drugs caused a highly significant reduction in weal size.

When the data obtained during the course of hydroxyzine were compared with those obtained with chlorpheniramine, the results were as shown in Table 2.

It is therefore clear that in three of the four test conditions the reduction of weal size with hydroxyzine was significantly greater than that with chlorpheniramine.

Symptomatic relief

When taking hydroxyzine twenty-nine of the thirty-three patients obtained relief of itching and when taking chlorpheniramine sixteen of the thirty-three patients had some relief from itching.

Side-effects

Five patients complained of drowsiness and one of lack of concentration whilst driving when on hydroxyzine, and two complained of excessive tiredness whilst taking chlorpheniramine.

DISCUSSION

It seems clear that the use of hydroxyzine in patients with symptomatic dermographism is of practical value in their management. Some patients complained of minor drowsiness in the schedule used in the tests, and it is now our common practice to give a dose of hydroxyzine pamoate of 25 mg in the morning and 50 mg at night. The observations recorded are of considerable interest in showing that in common clinical dosage hydroxyzine although a mild antihistamine usually caused a greater reduction in weal size than a commonly used antihistamine. It does not seem likely that this is due to the sedative effects and the true pharmacological explanation will have to await further work.

TABLE 3

Load (g/cm²)	Mean difference (back control–arm control) (mm)	P
3650	+0·82	0·001
7400	+1·36	0·001

Although it has been recognized that the size of weals varies considerably from site to site and that areas usually exposed do not react as much as covered areas (Kirby *et al.*, 1971), this point needs stressing if comparison of weal sizes are to be made. It is interesting that in the thirty-three patients described, a comparison of the weal size prior to treatment, on the forearm (compared) with that on the back gave the figures shown in Table 3.

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The use of plastic material for the protection of patients with severe photodermatoses

JOYCE HERD, I.STURROCK AND W.FRAIN-BELL

Department of Dermatology, University of Dundee

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SUMMARY

The use of blinds made of a semi-clear yellow plastic material has been found helpful in the management in hospital of patients with severe photodermatoses.

The reaction of the skin to light may vary in severity from a mild sunburn erythema to the severe and disabling responses seen in actinic reticuloid (AR) (Ive *et al.*, 1969). The alleviation of the distress experienced by subjects with AR is often difficult and usually requires strict avoidance of light or the systemic administration of corticosteroid drugs in maintenance amounts greater than that which can be given with safety. Also, the more severe examples of polymorphic light eruption (PLE), 'photosensitivity' forms of drug reactions and photocontact dermatitis may require from time to time maximum protection from short and long ultra-violet radiation or both. Although the action spectrum in AR is not restricted to the ultra-violet wavelengths (290–400 nm) but may also involve visible light from 400 to 600 nm, the increased ultra-violet sensitivity, particularly to the short wavelengths (<320 nm), probably plays a relatively large part in the abnormal skin responses. At the same time, the severity of skin response is such that intolerance to known light screening agents is such that their use is impracticable in the acute phase. Attempts to determine the action spectrum with appropriate phototesting procedures may be either impossible on account of the extreme reactivity of the skin or alternatively may lead to false conclusions. For these various reasons, and in view of the report by Daniels (1961) on the screening properties of certain substances, we constructed special blinds made from a plastic material, Uvethon, and our preliminary impression from their use in a dermatology ward over the last 6 months is that they assist the treatment and investigation of such patients.

MATERIALS AND METHOD

The blinds were made from a semi-clear yellow plastic material, Uvethon 'Y' (M & B Plastics Ltd, 22–25 Eastcastle Street, London W1N 8LL), which prevents exposure of the patient within the cubicle to ultra-violet radiation and visible light below 470 nm as can be seen from the transmission characteristics shown in Fig. 1. The blinds are fixed to the ward roof and can be raised or lowered as required (Fig. 2).

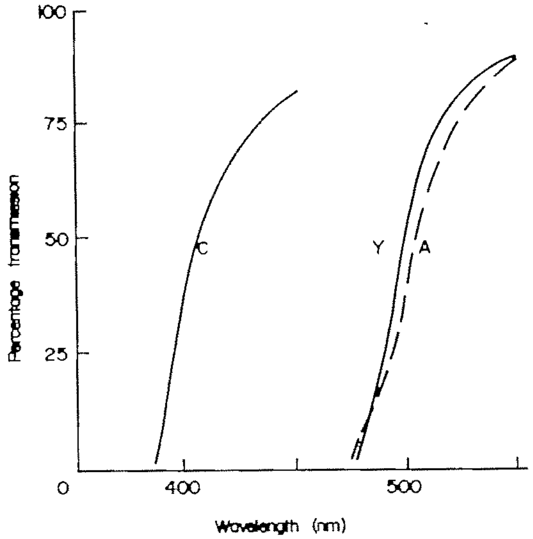

FIGURE 1. Transmission characteristics (SP8000) for each of the three plastic materials, Uvethon 'C', 'Y', 'A'.

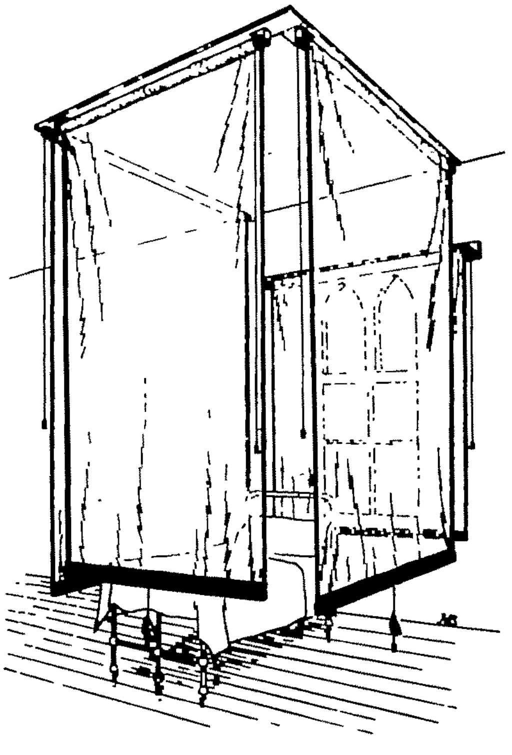

FIGURE 2. Bed protected by blinds of plastic material, Uvethon 'Y'.

DISCUSSION

We chose to use Uvethon 'Y' which is yellow in colour rather than Uvethon 'A' which is amber, on account of greater patient acceptability. Although Uvethon 'C' has the advantage of being colourless it will only screen off ultra-violet irradiation whereas the 'Y' and 'A' forms will also protect the skin from visible light up to 470 nm and this is of particular advantage in the care of patients with a broad action spectrum such as is found in AR. The Uvethon 'C' material would probably be sufficient for most patients suffering from PLE, drug reactions, or photocontact dermatitis. It remains to be seen whether the use of such material has a part to play in the domiciliary treatment of patients with severe reactions to light. Our impression is that its use has certain advantages in the study and hospital care of such patients.

ACKNOWLEDGMENTS

We are grateful to Miss M. Benstead, Medical Artist, Faculty of Medicine, University of Dundee, for the drawing shown in Fig. 2, and to Mr T. King, Department of Medical Photography, Dundee Royal Infirmary.

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Case Reports

Toxic epidermal necrolysis associated with pentazocine therapy and severe reversible renal failure

JOHN A.A.HUNTER AND ALEX M.DAVISON

Department of Dermatology, University of Edinburgh and Medical Renal Unit,
The Royal Infirmary, Edinburgh

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SUMMARY

A 62-year-old man developed toxic epidermal necrolysis 8 days after starting treatment with pentazocine. Fluid loss through the skin caused severe uraemia (blood urea 420 mg/100 ml) which was rapidly reversible after prompt parenteral fluid and electrolyte replacement.

Lyell described the features of toxic epidermal necrolysis (TEN) in 1956. Since then much interest has been focused on this striking reaction pattern and it is now generally accepted that the picture is usually due to a drug reaction or a staphylococcal infection. Recent research has gone a long way towards probing into the mechanisms at work in the latter instance (Kapral & Miller, 1971; Arbuthnott *et al.*, 1972; Melish, Glasgow and Turner, 1972) and a toxin has been isolated. At present much less is known about the pathogenesis of drug induced cases which tend to occur more often in adults and which are associated with a mortality of about 40% (Schuppli, 1970).

A recent leading article (*British Medical Journal*, 1971) expressed the view that there is no shock such as would be inevitable in a similar burn. However, others have considered that the high mortality is most likely due to fluid loss producing hypovolaemia and shock and secondary bacterial infection of the denuded areas (Braun-Falco, 1970). The case presented here seemed worthy of recording as it was associated with clinical shock complicated by severe but reversible uraemia. Circumstantial evidence strongly suggested that the drug pentazocine was responsible even though there appear to be no previous reports of such an association. A satisfactory outcome followed early and adequate parenteral fluid replacement.

CASE HISTORY

The patient, a 62-year-old retired male clerk gave a long-standing history of neck pains. A myelogram confirmed the clinical diagnosis of cervical spondylosis in February 1970 and the pain initially responded to treatment with dextropropoxyphene hydrochloride and paracetamol (Distalgesic). However, due to increasing severity, this was replaced (27 March 1970) by pentazocine (Fortral) 50 mg 4 hourly subsequently increased (2 April) to 75 mg, 4 hourly. On 3 April the patient noticed a blue coloured rash around his lips as well as tenderness in the mouth and 2 days later the skin began to peel off in sheets on his trunk and limbs. He was advised to discontinue pentazocine and chlorpheniramine maleate 4 mg 6 hourly was prescribed, but 24 h later his condition had deteriorated to such an extent that he was admitted as an emergency to the Department of Dermatology, The Royal Infirmary, Edinburgh.

On admission he was disorientated and clinically shocked with cold cyanotic extremities and a blood pressure of 105/60 mmHg. There were widespread sheets of desquamation on his trunk and limbs especially over pressure areas. The raw areas exuded copious amounts of serous fluid and the surrounding skin was erythematous, indurated and extremely tender. The Nikolski sign was so positive that it was difficult not to rub off skin when turning or moving the patient. There was extensive purpura affecting the intact skin of the lower limbs and there were crusted bloody areas on the buccal surface of the lips, buccal mucosa and soft palate. There was bilateral purulent conjunctivitis. The clinical picture was characteristic of TEN and biopsy confirmation of the diagnosis was deemed unnecessary. He was immediately given hydrocortisone 100 mg i.m. followed by Cloxacillin 500 mg q.d.s., prednisolone 40 mg daily and local treatment included framycetin eye drops, gentian violet and a spray containing oxytetracycline and hydrocortisone (Terra-Cortril) as well as careful turning and nursing on a ripple mattress.

Relevant investigations were as follows: Hb 14·6 g/100 ml, WBC 16,800/mm^3 with 89% neutrophils many of which showed toxic granulation, blood urea 430 mg/100 ml, sodium 129 mEq/l, potassium 3·6 mEq/l, chloride 83 mEq/l, bicarbonate 22 mEq/l, urinary sodium 13·6 mEq/l and urinary osmolality 480 mosmol/kg. *Staphylococcus aureus* and beta-haemolytic streptococci were isolated from the chest wall. Staphylococcal subcultures revealed two strains, neither of which had expressions in Group II. Blood cultures were repeatedly negative; standard liver function tests and serum protein electrophoresis were normal. An X-ray of the neck revealed persistence of dye from the previous myelogram.

During the initial 24 h he passed only small volumes of urine containing microscopic amounts of blood though after catheterization 1500 ml of sterile urine were obtained. In view of the elevated blood urea and clinical hypovolaemia he was transfused with 1 litre of plasma and 2 litres of normal saline and was encouraged to take oral fluids attaining a total intake of 5·5 litres in the subsequent 16 h. His blood pressure rose to 140/70 mmHg and the urine output increased satisfactorily. A high fluid intake was maintained and the blood urea fell rapidly and the electrolytes returned to normal values. Two weeks after admission his blood urea was normal and his creatinine clearance 108 ml/min with no haematuria or proteinuria.

The skin responded satisfactorily to treatment though the lesions over pressure areas were slow to heal. Three weeks following admission prednisolone therapy was discontinued after gradual reduction in dosage. At this time, and subsequently, his neck and shoulder pains have been adequately controlled with mefenamic acid 250 mg 6 hourly and no further skin or renal complications have arisen.

DISCUSSION

The differential diagnosis of TEN has been discussed by Wilkinson (Wilkinson, 1970) and we would certainly agree with him that the syndrome is usually so distinctive that the diagnosis is immediately evident. The oral signs in this case resembled those of erythema multiforme though the scalded looking areas on the trunk and limbs were clearly different. Lyell (Lyell, 1967, 1969) has pointed out that this resemblance appears to be common in those cases of TEN induced by drugs.

In this case the reaction followed therapy with pentazocine. It is possible that either a staphylococcal infection (conjunctivitis) or a contrast medium containing iodine (myelogram) could have been responsible for initiating the reaction. However, the patient firmly maintained that the purulent conjunctivitis developed *after* the rash and the staphylococci isolated were not of the phage types incriminated in the aetiology of this condition (i.e. not phage Group II type 3A, 3C, 52 or 71). According to present knowledge, and assuming the organisms isolated were representative, this would make it unlikely that this instance of TEN was connected with the staphylococcal infection (Lyell, 1971). The absence of recrudescence following the cessation of steroid therapy in the continuing presence of Myelodil makes the second possibility also unlikely. The patient did not admit on repeated questioning to taking any other drugs. To our knowledge pentazocine has not previously been associated with TEN (Bandmann, 1970) although other dermatological side-effects such as inflammation, fibrosis and ulceration have been reported to occur at injection sites (Parks, Perry & Muller, 1971).

Lyell (1971) mentioned that of the 128 cases that he had details, eight had shown some disturbance (usually raised blood urea) of renal function. There are two likely mechanisms for this. Loss of fluid into indurated skin and from denuded areas can be copious and may produce hypovolaemia, uraemia and oliguria (Beyer *et al.*, 1962; Manganotti & Silvestri, 1963; Hoigné 1970). It also seems probable that some of the patients may develop a concomitant glomerulonephritis as part of the general reaction pattern (Lyell, 1956; Beyer *et al.*, 1962; Manganotti & Silvestri, 1963; Krumlovsky, 1972). This is reflected clinically by haematuria, proteinuria and degrees of uraemia, all of which may or may not be reversible. To date, pathological reports of the kidney in TEN are sparse but some (Lyell, 1956; Manganotti & Silvestri, 1963; Krumlovsky, 1972) have confirmed a proliferative glomerulonephritis involving mainly the mesangial regions. The problem is at present being reviewed by the Renal Study Group at Passavant Memorial Hospital, Northwestern University Medical Center, Chicago (Krumlovsky, 1972).

In this case the uraemia was most likely explained by hypovolaemia secondary to fluid loss into indurated skin and from denuded areas; this being supported by the finding of a low urinary sodium and a dramatic response to volume replacement with plasma and saline. It has been pointed out (Schuppli, 1970; Hoigné, 1970) that prompt and adequate parenteral fluid and electrolyte replacement is essential, particularly as such disturbances are likely to be factors influencing the mortality (Bailey, Rosenbaum & Anderson, 1965; Braun-Falco, 1970). We know of no case where recovery occurred after such a degree of uraemia.

ACKNOWLEDGMENTS

We would like to thank Dr A. Lyell for his encouragement to report this case and to Dr A. G. Grant Peterkin for his permission to do so. Dr J. P. Arbuthnott kindly arranged for the phage typing of the staphylococcal isolates.

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Toxic epidermal necrolysis provoked by barbiturates*

G. STÜTTGEN

Free University of Berlin, Rudolf Virchov Hospital, Berlin

Accepted for publication 3 August 1972

SUMMARY

The case is reported of an elderly woman in whom toxic epidermal necrolysis, originally provoked by sensitivity to one barbiturate, primidone, subsequently recurred after the administration of another, phenobarbitone.

Some patients with toxic epidermal necrolysis (TEN) give a history of earlier drug eruptions of various types. Then on taking one of these same drugs or a new drug TEN suddenly develops. If the responsible drug or a related substance is taken, even after a long interval, the TEN recurs in increased severity. The observations of Lyell (1967) and of Faninger (1961) have illustrated this sequence of events, but this is only one of many possibilities.

CASE REPORT

The patient was a woman aged 70 who had been treated for trigeminal neuralgia for 10 years. As she

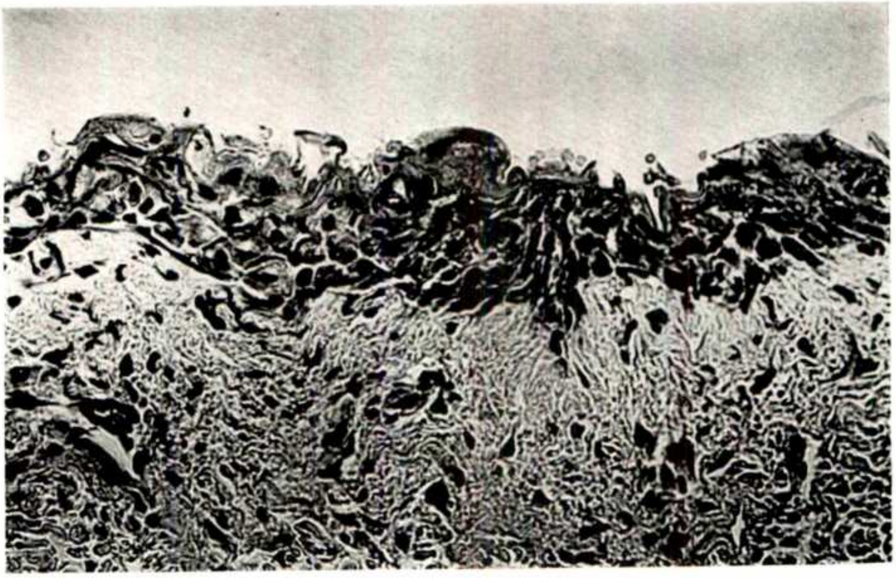

FIGURE 1. Histological appearance on the fifth day of the first eruption induced by Mylepsin.

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* Paper presented to the British Association of Dermatology, London, July 1972.

TABLE 1. The precipitating stimuli of TEN in the case of drug allergy

Specific eliciting drug → Interval (hours–days) — TEN → [months–years] → Specific eliciting drug → TEN recurrence

Preparatory phase—non-specific
(1) Infections (virus, bacterial)
    Endotoxin–epidermal complex
    epidermal damage
(2) Drug eruptions of various type
    Allergen–epidermal complex
(3) ?

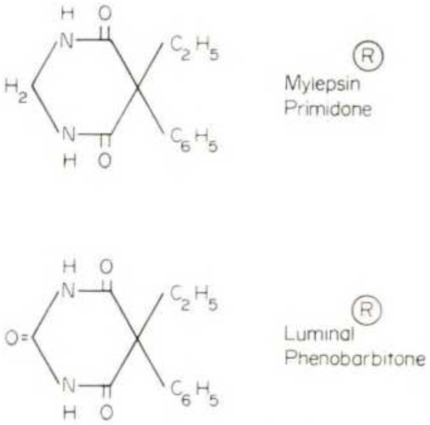

FIGURE 2. Chemical formulae.

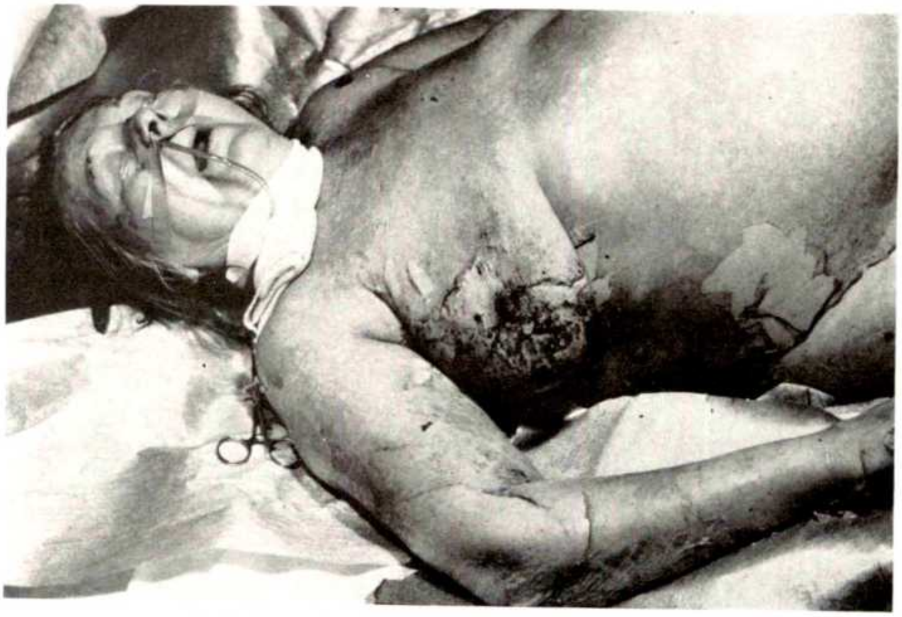

FIGURE 3. The recurrence after phenobarbitone.

had become allergic to phenothiazines and carbamal-dibenzoacepin, primidone (Mylepsin), a barbiturate was prescribed. She developed acute TEN. Treatment with intravenous prednisolone was effective and she recovered completely within 2 weeks.

A year later 300 mg of phenobarbitone was inadvertently prescribed for a relapse of the trigeminal neuralgia. Within 24 h TEN had developed and it reached its maximum extent in 2 days. Corticosteroids equivalent to prednisone 200–500 mg daily for 14 days suppressed the bullae and the Nikolsky phenomenon. However, fever up to 40° C persisted, with hypotonia and somnolence, and the patient died 3 weeks later of sepsis and profuse gastro-intestinal haemorrhage.

At autopsy verrucous and ulcerative endocarditis was found. The adrenal cortices were atrophic and depleted of lipid. There were haemorrhages in skin, pleura and viscera. The pathologist attributed the adrenal changes and the endocarditis to the corticosteroids.

Investigations

Skin scarification with barbiturates, the lymphocytic transformation test and direct and indirect fluorescence microscopy were all negative.

DISCUSSION

Despite the negative immunological findings, the evidence suggests that this was an allergic reaction to two related barbiturates.

In treatment the fluid and electrolyte balance must be maintained as in the management of burns. Despite their hazards the corticosteroids have proved their value, and given in reducing doses may prevent relapses.

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Unusual skin eruption with eosinophilia associated with hyperthyroidism

J.S.PEGUM AND KATHERINE GRICE

Department of Dermatology, The London Hospital, Whitechapel, London E.1

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SUMMARY

An unusual skin manifestation of Graves disease is described which varied from exfoliative dermatitis to toxic erythema and to more circumscribed 'fixed urticaria'. There were also lichenoid lesions. Persistent pyrexia, eosinophilia and a raised ESR were also present. Other features of hyperthyroidism included myopathy and arthropathy. It is suggested that an immunological mechanism could account for the unusual manifestations in this patient.

We describe a patient with an unusual widespread skin eruption associated with thyrotoxicosis due to Graves' disease. The eruption persisted for 6 months, and only finally cleared when the hyperthyroidism was successfully treated by surgery.

A variety of skin lesions have been described with hyperthyroidism. These include generalized pruritus or erythema, chronic urticaria and angioneurotic oedema, hyperhidrosis, vitiligo and increased pigmentation, increased incidence of eczema, onycholysis and koilonychia, diffuse alopecia, canities, and hirsutism, pretibial myxoedema and acropachy (Luria & Asper, 1958; Readett, 1964; Barrow & Bird, 1966; Ochi & DeGroot, 1969; Miller, Ashkar & Jacobi, 1970; Nixon & Samols, 1970; Isaacs & Ertel, 1971). Lupus erythematosus has also been reported in association with thyrotoxicosis (Papadopoulos, Rouben & Maher, 1969).

CASE REPORT

V.H. a girl aged 19, a clerk in the Women's Royal Naval Service, with no previous or family history of skin eruptions or atopy.

History of clinical findings (January–April 1971)

In January 1971, 1 week after a sore throat associated with pruritus of the neck, she developed swelling of the eyelids and an erythematous papular eruption on the dorsa of the hands which spread to involve the flexor surfaces of the upper and lower limbs and persisted for 3 weeks. The lesions cleared for 1 week then re-appeared and spread to all areas of the body including the vulva, pinnae and external meatus. She was admitted to the Royal Naval Hospital, Haslar in the care of Surgeon Captain Scutt, at which time her ears were discharging, there was marked generalized pruritus, confluent erythema and oedema of the face, generalized lymphadenitis, persistent pyrexia and eosinophilia (1000–3000/mm^3). The liver was enlarged 3 cm below the costal margin, the spleen was not palpable. Exfoliative dermatitis developed and she was therefore given prednisolone, 60 mg/day with marked improvement, with initial antibiotic cover of Cloxacillin and Procaine penicillin. No drugs had been given prior to this. This was changed to ACTH 20 units on alternative days. Various diagnoses were suggested at that time, including erythema multiforme, photodermatitis, seborrheoic dermatitis, dermatomyositis and exfoliative dermatitis. In April 1971 she was transferred to the London Hospital.

Clinical findings on admission

Since the onset of her illness she had lost 6 kg in weight. There was aching of the muscles of the shoulders, upper arms and thighs with some weakness of the quadriceps and deltoids. Pain was present in the joints of the hands, feet, ankles and wrists. Hair had been lost from the scalp, there was marked tachycardia, 130/min, the right lobe of the thyroid was just palpable and there was a fine tremor of the outstretched hands. Minimal lid-lag was present, but there was no proptosis.

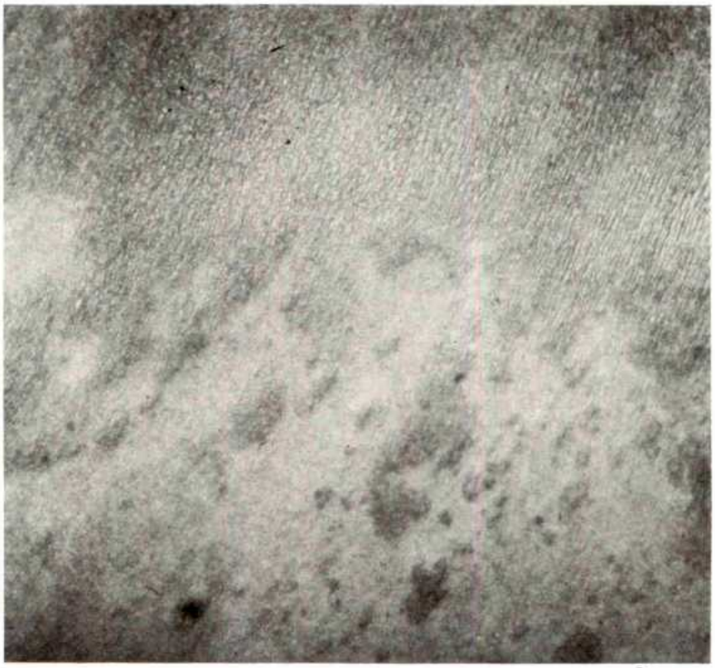

FIGURE 1. Toxic erythema-like eruption on the abdomen.

Skin eruption

There was diffuse erythema with some scaling and oedema of the face, this being most marked around the eyes. The eruption on the limbs and trunk was that of a 'toxic erythema' (Fig. 1) being polymorphic and pruritic with scattered fixed urticarial lesions. Occasional target like lesions were seen on the backs of the fingers, and a lichenoid eruption appeared on the abdomen. The extent of the erythema varied from day to day.

Initially the backs of the hands, flexor surfaces of the forearms, palms, soles (Fig. 2) and anterior surfaces of the thighs were affected, but on ceasing steroid therapy the erythema became diffuse, the

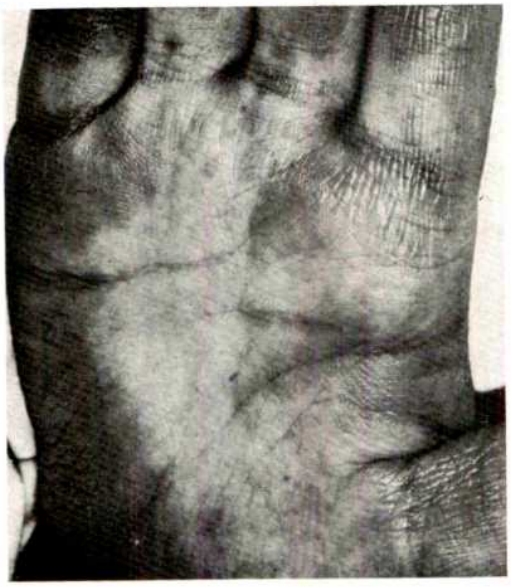

FIGURE 2. Patchy erythema and scaling of the palm.

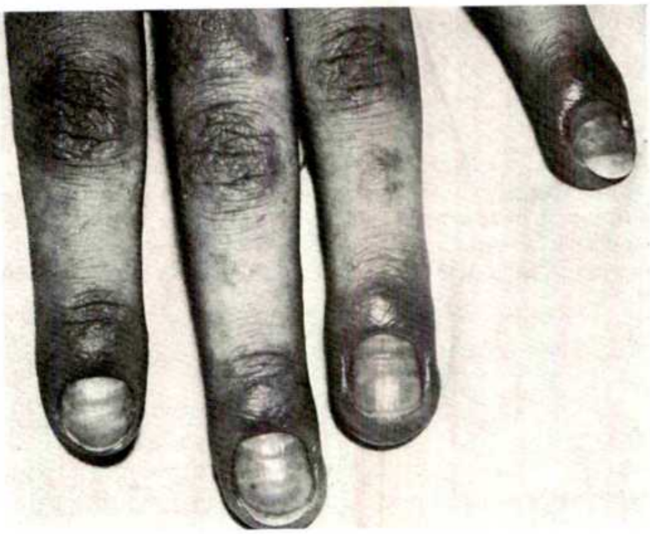

FIGURE 3. Nail folds, swollen shiny, and red with dilated capillaries. There are also subungual haemorrhages and erythematous papular plaques on the back of the fingers.

urticarial lesions more widespread, and there were subungual haemorrhages of several toe nails and thickening of the nail folds of the fingers (Fig. 3). Chlorpheniramine maleate, 16 mg/day and trimeprazine tartrate, 30 mg/day helped the irritation, but had no effect on the fixed urticaria.

PROGRESS AND INVESTIGATIONS

In April, ACTH was stopped. There was persistent tachycardia (100–130/min), eosinophilia, raised sedimentation rates (ESR) and increased serum IgG and IgM (see Table 1). There was no recurrence of pyrexia. Electromyograms showed a gross myopathy with normal motor conduction. Radio-iodine uptake was in the hyperthyroid range at 3 h 62% (normal 6–29%) and at 24 h 68% (normal 20–55%). The thyroid scan showed both lobes to be small. The most active region was in the larger right lobe which gave a very high maximum count rate. The protein-bound iodine was 11·3 μg/100 ml (normal 3·5–8).

TABLE 1. Laboratory data (other than that shown in the text)

Sedimentation rate (Westergren) mm/h (range) 28–62

Eosinophil count/mm^3 (range) 1744–6232

WBC/mm^3 (range) 7000–14,000

Serum transaminase SGOT (range) 42·5–48 units/ml

Creatinine phosphokinase (range) 1·1–3·0 sigma units/ml (normal < 14)

Serum aldolase (range) 3·4–4·1 milliunits/ml (normal < 4)

Serum proteins 6·5 g/100 ml (albumen 3·2, globulin 3·3)

Serum calcium 8·7 mg/100 ml

Serum, bilirubin, alkaline phosphatase, thymol turbidity, zinc sulphate turbidity, Cl, bicarbonate, Na, K, urea: normal

Serology, WR, VDRL, Reiter protein CFT, GCFT, *S. typhi*, *S. paratyph.*, non-specific *Salmonella*, brucella abortus, trichinella CFT: negative

Monospot Paul Bunnell, Hela cell culture and sucking mice, Coxsackie A & B, toxoplasma dye test: negative

Chest X-ray and barium swallow normal, liver and spleen scan, liver 3 cm below costal margin

LE cells not found. Hb, RBC: normal

There was no thrombocytopenia nor increase in the number of lymphocytes, as has been reported in hyperthyroidism by Lamberg *et al.* (1971)

Serum immunoglobulins IgG 240% (normal 48–168) IgM 450% (normal 50–180) IgA 165% (normal 50–172)

Histology (Dr J. Landells)

Skin biopsies from thigh and abdomen showed histiocytic infiltration around vessels and appendages, but no vasculitis. There was some extravasation of red blood cells in one biopsy with a few perivascular lymphocytes, histiocytes and melanophores in dermis, but no eosinophil leucocytes were seen. A few mast cells were present, but neither they nor their granules were in excessive numbers. The basal membrane was broken up in one or two foci in one biopsy, but there was no nuclear fragmentation (Fig. 4). Skin biopsy from the foot showed minimal perivascular lymphocytes only

Cervical node showed distension of gland sinuses by macrophages without inflammatory oedema and moderate increase in germinal centres. The reaction was consistent with the presence of non-specific damage to the skin drained by the node

It was felt that the clinical findings could be explained by hyperthyroidism and she was started on pre-operative potassium iodide. Whilst on this medication the skin eruption improved.

Sub-total thyroidectomy was performed in June by Mr J. E. Richardson. The histological changes were those of Graves' disease. The thyroid showed diffuse parenchymal hyperplasia with activity varying from markedly excessive to normal in different lobules. There was a mild focal lymphocytic thyroiditis.

Postoperative progress

The immediate postoperative period was stormy with marked tachycardia up to 140/min, pyrexia 38·5–40° C, extreme agitation and a marked worsening of the skin eruption. There was a fresh outbreak of the 'fixed urticaria' lesions in all areas with worsening of the facial erythema, periorbital oedema and scaling of the scalp. The urticarial lesions were clinically identical with ordinary urticaria but individual lesions persisted for up to a week. These manifestations gradually subsided and she was discharged home 2 weeks after thyroidectomy with the skin practically clear. Since then all the signs and symptoms have subsided and she has remained well.

Further laboratory and histological data are shown in Table 1 and Fig. 4.

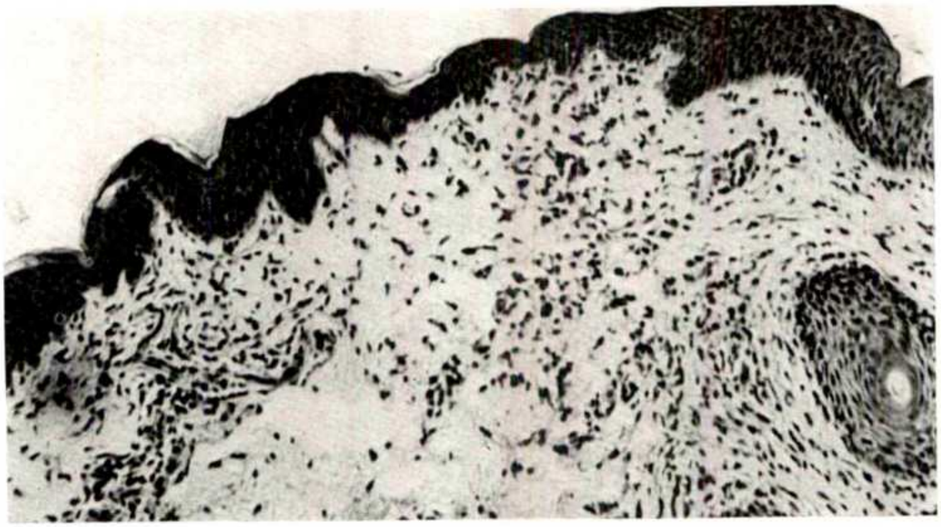

FIGURE 4. Skin biopsy from abdomen Perivascular histiocytic infiltration and some upper dermal oedema (H & E, × 90).

Special investigations

Skin tests. Skin tests were performed pre-operatively with 0·1 ml tri-iodothyronine (T_3) and thyroxine (T_4). No positive responses were seen at the site of the injection at 0·5, 1, 4, 8, 24 or 48 h to either T_3 (50 μg/ml) or T_4 (100 μg/ml). However, 4–8 h after the skin tests, the skin lesions flared up, the ears started discharging and the erythematous-urticarial lesions became widespread. Tests were also performed with the thyroid tissue removed at operation. Two preparations were used; centrifuged filtered extracts of thyroid tissue and a filtered and autoclaved extract. No positive responses were obtained following intradermal injection of 0·1 ml of a 1 in 40 dilution of each preparation, either immediately or up to 48 h later. Three months after operation attempts were made to provoke the skin eruption by giving in turn by mouth: T_3 20 μg, T_4 50 μg and dextrothyroxine 2 mg. No effects were noticed.

Cold studies. Pre-operatively there was marked urticaria which lasted for some hours after the skin cooling produced by PCL spray (dichlorodifluoromethane 50%, trichloromonofluomethane 50%). When repeated 3 months after operation the test was negative and significant cold agglutinins were not detected.

Leucocyte migration test. Migration inhibition studies were performed to try and detect the presence of delayed hypersensitivity to thyroid gland constituents (Brostoff, 1970). Washed buffy coat leucocytes were placed in capillary tubes and allowed to migrate in the presence of antigen. The presence of migration inhibition would be indicative of cell sensitivity to the relevant antigen. However, no migration inhibition was seen with either T_3, T_4 or thyroglobulin.

Antibody studies. The microsomal fluorescent thyroid antibody was weakly positive, but the following were negative for the presence of antibody: Thyroglobulin tanned red cell test (TRC), microsomal complement fixation test (CFT), gastric parietal cell, ANF, mitochondria and smooth muscle.

Precipitating antibodies to thyroglobulin were demonstrated in the patients serum. The method used was that of Feinberg *et al.* (1969).

DISCUSSION

It was some time before it was realized that this patient's skin eruption was due to Graves' disease. The intensely irritating eruption varied from generalized exfoliative dermatitis via toxic erythema to a curious fixed urticaria which was unresponsive to antihistamines. Unusual also in Graves' disease was the persistent eosinophilia (up to $6252/mm^3$) and the raised ESR. Some of the other manifestations of hyperthyroidism in this case were also unusual although well recognized. These included myopathy, arthropathy and lymphadenopathy.

The relationship between thyrotoxicosis and many of its dermatological manifestations is obscure. It has been suggested that the urticaria and pruritus in hyperthyroidism might be caused by accumulation of kinins (Isaacs & Ertel, 1971).

In the present case the mechanism of the skin lesions could have been immunological. Supporting this view was the presence of precipitating antibodies to thyroglobulin and the generalized flare up of the skin 4–8 h after the intradermal injection of T_3 or T_4. These both suggest an immunological reaction of the serum sickness type due to circulating immune complexes. If a thyroid hormone were the antigen it would be possible to explain the improvement on potassium iodide therapy. This treatment would have the effect of reducing the hormone concentration in the circulation thus allowing the immune complexes to clear. The stormy postoperative period could have been due to the systemic release of large amounts of thyroid gland components in the face of circulating antibody thus precipitating an acute serum sickness reaction as well as a thyrotoxic crisis. The lack of local Arthus reactivity on skin testing is surprising in the face of the systemic reaction engendered by the injection. The leucocyte migration inhibition test was negative to T_3, T_4 and thyroglobulin as were the skin tests to the same substances as well as extracts of the patient's own thyroid tissue.

Unfortunately definite evidence that the skin lesions were due to immune complex deposition was not obtained. Confirmatory investigations would have been the demonstration of immunoglobulin deposits and altered complement components (β1c) in the skin and possibly the presence of circulating antiglobulins.

We have not been able to find a precisely similar case described in the literature but a case shown by Kurban & Rayyis (1970) to the Dowling Club meeting in Beirut had some similar features. Their patient had a persistent pruritic erythematous eruption in the light exposed areas, with some pruritus of the covered areas, associated with hyperthyroidism. The scaling erythematous lesions of the face were very similar to those of our case. The lesions subsided when she was treated with ^{131}I. She subsequently became hypothyroid and the skin condition relapsed after one dose of thyroxine. Intradermal testing showed a positive reaction to T_4 and circulating antibody to her own and synthetic thyroxine was found in high titre.

ACKNOWLEDGMENTS

We are grateful to Surgeon Vice-Admiral Sir Eric Bradbury for permission to report this case. We thank Surgeon Captain J. M. Cliff and Surgeon Captain R. W. B. Scutt. Dr J. Perrin kindly helped with skin tests. We would also like to thank Dr Jonathan Brostoff for his help with the various immunological investigations. The London Hospital and Schering A. G. Berlin provided financial support for K.G.

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Comment

POLYMERIC COLLAGEN

The secrets of the structure and metabolism of collagen are yielding to continuing investigation that is now in a most intriguing phase. The advances include the deciphering of the complete structure of collagen and much of the pathway of its biosynthesis, which are lucidly described in the recent review by Grant & Prockop (1972) which will repay reading in full. Furthermore, it is now known that the extracellular collagen fibre is metabolically active (Bailey, 1968; Nordwig, 1971) and not inert, as was thought until a decade ago. Thus there are numerous opportunities for biochemical abnormalities in collagen.

The most important characteristic of the collagen molecule is its intrinsic ability to aggregate and so form fibres (Veis, 1967; Kuhn, 1969; Steven, 1972) and quite small modifications to the basic structure of the molecules probably determine whether they will aggregate into an extensive feltwork of fibres (e.g. dermis) or delicate overlapping sheets (e.g. cornea) (Schofield, Freeman & Jackson, 1971; Segrest & Cunningham, 1971; Kefalides, 1972).

Collagen's other important property is its ability to become polymerized by forming a series of chemical cross-links between the individual molecules of a fibre (Steven, 1972; Fowler & Bailey, 1972). The role of these cross-links in stabilizing connective tissue was realized over 10 years ago when lathyrism, a disease of bowed bones, weak skin and often rupture of the aorta, was shown to result from a defect in cross-link formation (Bornstein, 1966). Attempts to utilize cross-link inhibition for therapeutic purposes have already been made. Penicillamine, which has a similar though not identical effect (Deshmukh, Deshmukh & Nimni, 1971) to β-amino proprionitrile (BAPN), the substance causing lathyrism, has been used for these studies. Though safer agents need to be developed, penicillamine is being studied in the treatment of systemic sclerosis and rheumatoid arthritis (Harris & Sjoerdsma, 1966b; Zuckner *et al.*, 1970).

Several types of cross-link are now known, as are some of the enzymatic steps in their biosynthesis (Grant & Prockop, 1972). Connective tissue cells hydroxylate some of the lysine residues of the three peptide chains (α-chains) which intertwine to form the collagen molecule (Kivirikko & Prockop, 1972). In the fibres, some of the hydroxylysine and lysine residues are converted by a specific enzyme to an aldehyde (Layman, Narayanan & Martin, 1972) and these aldehydes can then react with lysines or hydroxylysines on adjacent collagen molecules to produce chemical cross-links between the molecules (Grant & Prockop, 1972; Fowler & Bailey, 1972). In this way weakly joined molecules in a fibre are converted to polymeric collagen.

These collagen polymers account for over 99% of the total collagen of mature individuals. Initially, the cross-links are unstable, but with increasing age more and more are converted to chemically and physiologically more stable forms (Bailey & Shimokomaki, 1971; Grant & Prockop, 1972).

The predominant cross-link varies in differentt issues. The dominant link in bone differs from that in mature skin but, interestingly, is similar to that of embryonic skin collagen and the collagen found in scar tissue (Davies & Bailey, 1972; Bailey, Peach & Fowler, 1970; Bailey and Robins, 1972; Forrest *et al.*, 1972).

A possible clinical application of this knowledge has been the detection of a reduced lysine content in the collagen of two siblings affected with severe scoliosis (Pinnell *et al.*, 1972).

Studies of collagen in disease states started after it was demonstrated that the amino acid hydroxyproline was sufficiently specific for collagen as to allow the total collagen content of dermis to be determined from measurements of the hydroxy-proline concentration. The small contribution to the dermal hydroxy-proline content made by elastin may be safely ignored in most conditions (Eastoe, 1967). Shuster and his colleagues have shown that total dermal collagen rises with age, varies with sex and at different biopsy sites (Shuster & Bottoms, 1963), falls with corticosteroid therapy (Shuster, Raffle & Bottoms, 1967) and in diseases such as systemic sclerosis (Black, Bottoms & Shuster, 1970) and osteogenesis imperfecta (Stevenson, Shuster & Bottoms, 1970).

Until recently, investigators of the structure of dermal collagen in disease have studied the small proportion of the total tissue collagen that can be extracted by concentrated neutral-salt or dilute organic acid solutions (Uitto, 1971; Harris & Sjoerdsma 1966a). Elevation of salt-soluble collagen has been observed in some diseases such as Marfan's syndrome (Laitinen *et al.*, 1968) and dermatomyositis and scleroderma (Uitto *et al.*, 1967). However, these fractions contain only the poorly cross-linked collagen which in health constitutes less than 5% of total adult collagen (Veis, 1967), and there has been a need for techniques which allow one to study the highly cross-linked (polymeric) collagen characteristic of mature dermis.

The cross-links of polymeric collagen can be studied directly, but present techniques require sophisticated analysis and even then are able to measure only the initially formed, chemically unstable cross-links (Bailey & Shimokomaki, 1971; Steven, 1972). An alternative approach has been possible since Steven (Steven, 1964, 1967, 1971; Veis *et al.*, 1970) demonstrated that the hitherto inaccessible polymeric collagen can be extracted from tissues. These techniques can be used on 75 mm^2 biopsies of human skin (Francis & Macmillan, 1971). The polymeric collagen extracted in this way is treated with a series of degradation procedures that give a measure of the numbers and types of cross-links present, even although the exact nature of the cross-links is not known (Steven, 1966; Francis & Macmillan, 1971). For example, the number of cross-links increase with age (Francis, Smith & Macmillan, 1972a). Such tests are easily applied and can later be extended by more complex and specific analysis of cross-links.

The cross-linking of skin polymeric collagen has proved to be abnormal in several diseases clinically affecting mainly internal connective tissue, such as osteogenesis imperfecta, homocystinuria and Werners syndrome (Francis, Smith & Macmillan, 1972b). Unstable dermal polymeric collagen was present in lesions of necrobiosis lipoidica, and also, in some patients, in their clinically normal skin (Francis & Macmillan, 1972).

Even the few results so far reported on the cross-linking of polymeric collagen in disease states have indicated the potential usefulness of such studies. They will identify in which diseases more complex cross-link and metabolic studies are needed and will likely prove of value for following the course of diseases and their response to treatment because they provide a measurement which is objective.

United Oxford Hospitals

D.C. MACMILLAN
M.J.O. FRANCIS

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British Journal of Dermatology (1973) **88**, 307.

Correspondence

IMMUNOGLOBULIN AND COMPLEMENT BEARING LYMPHOCYTES IN ECZEMA

SIR, Direct immunofluorescence studies of biopsies taken from positive patch tests (twenty) elicited by various antigens, revealed the presence of lymphocytes with membrane-bound immunoglobulins (Ig) in the round-cell infiltrates. The dominating class appeared to be IgD. Moreover, lymphocytes with membrane-bound complement (C_3) were noted.

Very recent findings, mainly stemming from animal experiments, indicate that membrane-bound Ig and C_3 are markers of the bone marrow-derived 'B'-cells, which are thought to be precursors of plasma cells.

The above mentioned observations led to an investigation of the occurrence of 'B'-cells in the peripheral blood in patients with various dermatoses and healthy controls.

For this purpose lymphocytes were separated from 20 ml defibrinated blood samples by the Isopaque–Ficoll techniques as described earlier (Böyum, 1968). The cells were then washed three times in Hanks' balanced salt solution. By a membrane-staining technique (Fröland & Natvig, 1972), fluorescein-conjugated monospecific antisera to the five main heavy-chain classes and complement (C_3) were reacted with the purified human blood lymphocyte suspensions for 30 min at 37°C. After incubation, cells were washed twice in isotonic NaCl solution and resuspended in 0·2 ml of the same solution. A conjugated anti-DNA antiserum was usually included as control. The smears for immunofluorescence (IF) were prepared according to the methods described by E. Szabò (personal communication).

Immunofluorescence examination was performed in suspension and in unfixed thin smears. In each case the percentage of Ig- and C-positive lymphocytes was determined by examination of the microscope field alternately in blue narrow-band epi-illumination for fluorescence and in conventional light.

In all patients (ten) with contact eczema, IgD was invariably found as the dominating class on lymphocytes with a mean of 36% and a range of 13·5–65%. The mean percentages and ranges of lymphocytes with C_3 on their membranes were about similar to those given for IgD positive lymphocytes.

In atopic patients (ten), however, IgE bearing lymphcoytes were dominating in peripheral blood, with a mean of 20% and a range of 11–25%, whereas the values of C_3 bearing lymphocytes were within the normal range.

In healthy controls (ten) the mean of IgD positive lymphocytes was 4·5% with a range of 1·5–10%, that of I–E positive cells 3·5% with a range of 0–5%, and that of C_3 positive cells 6% with a range of 2·5–10%.

These results are compatible with the view that 'B'-cells do play an important role in the pathogenesis of sensitization.

Department of Dermatology
University of Amsterdam
Binnengasthuis
Amsterdam

R.H. CORMANE
S. HUSZ*
F.F. HAMERLINCK

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* Visiting dermatologist from the Department of Dermatology, University of Szeged, Szeged, Hungary.

ABSENCE OF THE GLOXYLATE SHUNT IN THE SKIN OF MOUSE AND RAT

SIR, There have been sporadic reports in the literature indicating that the 'glyoxylate shunt' pathway may operate in mammalian skin. This idea was originally suggested by Halprin & Chow (1961) as a theoretical explanation of the high succinate levels observed in dog skin. Kohn & Laden (1962) showed that glyoxylate disappears (albeit very slowly) when added to homogenates of rat skin. They qualified their finding, however, with the comment that 'additional experiments will be needed to establish whether the glyoxylate which is disappearing is being metabolized via the glyoxylate shunt mechanism.' More recently Brown & Box (1968) reported that glyoxylate (measured as the 2:4-dinitrophenylhydrazone derivative) is formed by incubating rat-skin homogenates with isocitrate; unfortunately their attempts to obtain a positive identification of the glyoxylate were 'frustrated by the minute amounts of glyoxylate formed.'

The key reactions in the glyoxylate shunt pathway are:

$$\text{(1) isocitrate} \xrightarrow{\text{isocitrate lyase}} \text{glyoxylate} + \text{succinate}$$

$$\text{(2) glyoxylate} + \text{acetyl-CoA} \xrightarrow{\text{malate synthetase}} \text{malate.}$$

The malate may then proceed via oxaloacetate and phosphoenolpyruvate to pyruvate. Thus, this pathway is remarkable in that it offers the possibility of a quantitative conversion of fat to carbohydrates. Although this is of vital importance to organisms whose sole source of carbon is lipid or acetate (germinating seeds, various micro-organisms), mammalian tissues are in general unable to accomplish such a transformation. Clearly, therefore, the occurrence of the glyoxalate shunt in skin would be of the greatest theoretical interest, and might well explain certain anomalies in the manner in which epidermis metabolises carbohydrate (Mier, 1969).

Fluorimetric techniques are now available for the assay of Krebs' cycle intermediates which are both more specific and more sensitive than the colorimetric assays used previously. In particular, malate may be measured by the fluorescence of NADH liberated during its NAD-linked oxidation by malate dehydrogenase (Williamson & Corkey, 1969); in our hands the ultimate sensitivity is about 2×10^{-11} mol, with a reproducibility of better than 10^{-10} mol. We have recently applied this procedure to skin perparations in an attempt to investigate the conversion of glyoxalate to malate.

Homogenates were prepared from mouse or rat skin; in some experiments these were used directly, and in others they were centrifuged to yield a clear supernatant. Glyoxalate (1 μmol), acetyl-CoA (0·2 μmol) and the skin preparation (40 mg/ml) were incubated in a tris-Mg^{++} buffer (total volume 1 ml, pH 7·4) at 37°C. Samples were removed at intervals over a 20 min period, heated to 100°C, for 2 min, centrifuged, and 50 μl aliquots assayed for malate. The results were uniformly and conclusively negative; no malate synthesis was observed. The possibility that newly-synthesized malate was being utilized by the skin preparation was eliminated by a control in which the concentration of added malate (2×10^{-8} mol/ml) remained constant during the incubation period. In order to confirm that incubation conditions were appropriate for malate synthetase, additional experiments were conducted using an extract of bakers' yeast in place of the skin preparation; in these experiments vigorous synthesis of malate occurred, the reaction rate remaining linear for at least 20 min.

If the reproducibility of the malate assay is taken as 10^{-10} mol, is is seen that the upper limit for glyoxalate shunt activity is $2·5 \times 10^{-9}$ mol/min/g wet weight of skin. Since this is only about 1% of figures which have been reported for glycolysis, it is clear that the glyoxylate shunt does not play a significant role in the skin of our animals. The comment of Brown & Box (1968) that 'details of diet are highly relevant' seems intrinsically unlikely; we are unaware that the induction of an enzyme system has ever been demonstrated in skin. A more probable explanation of the occasional appearance of glyoxylate might lie in the skin flora; micro-organisms adapted to the metabolism of surface lipids should certainly possess an active glyoxylate shunt.

Department of Dermatology
University of Nijmegan
The Netherlands

PAUL D. MIER
JOSÉ J. M. A. VAN DEN HURK

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TREATMENT OF PSORIASIS WITH VITAMIN A

SIR, I thought I might add some further information concerning the treatment of psoriasis with vitamin A, not given in a recent letter by Dr Whittle (Whittle, 1972). We ourselves certainly did follow up our findings on mouse tail epidermis with full prolonged clinical trials both at U.C.H. and at Luton with the co-operation of Dr Mary Witham. These results were fully published in a monograph together with the experimental animal results (Jarrett & Spearman, 1964). These were not carried out on a double blind basis because Dr Whittle and his colleagues at Cambridge had kindly agreed to undertake this task (Whittle *et al.*, 1961). Seventy-four patients were in the series and fifty of these were treated with the topical application of a cream containing a combination of vitamin A and triamcinolone. Of these fifty, seventeen were completely cleared, twenty were markedly improved, seven improved, and six relatively unaffected. None were made worse with this combined treatment, but with vitamin A alone irritant effects did occur. The rationale of this treatment was discussed more recently in this journal (Jarrett & Spearman, 1970).

Our latest work has been to compare experimentally the effects of different isomers of vitamin A including retinoic acid, and this material will shortly be submitted for publication. It would appear from the results so far obtained that there is little difference between the various forms of vitamin A as to their action on the induction of a granular layer in mouse tail-scale epidermis, the stimulation of epidermal mitotic activity, and the production of irritant effects. It was because of the last two effects that we decided to combine the vitamin A with fluorinated steroid which reduces both these actions.

The fact that cases relapse after ceasing treatment does not necessarily invalidate any method of therapy for psoriasis. In fact, with such a notoriously fluctuant condition, relapse on stopping treatment may be taken as confirmation that the improvement was due to the treatment and not to a spontaneous natural remission.

In support of this, the whole integument of psoriatic patients appears to be abnormal, as shown by its response to a standard stimulation (Jarrett & Reid, 1967) and by more recent work on the quantitation of acid hydrolases in apparently normal skin of psoriatics (Jarrett, 1973). These findings would suggest that at the present time we cannot expect to develop a treatment for this disease which will effect a permanent cure, but at the best we can only hope to maintain the patient free from lesions as long as the treatment is applied or until he enters one of the natural remissions many of these patients enjoy.

A. JARRETT

Dermatology Department
University College Hospital Medical School
University Street
London W.C.1

REFERENCES

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Book Reviews

Die klinische Electromyographie in der Differentialdiagnose von Neuro- und Myopathien. KARL-HEINZ PUFF (1971). Berlin, Heidelberg, New York: Springer. Pp. 84+VIII, 12 illustrations, 6 tables. Price £6.70.

This is an analysis of 3000 electromyographs in 1200 patients suffering from chronic neuromuscular disorders or myopathy in 5 years.

It would be of considerable interest to neurologists and physicians engaged in the diagnosis of disorders of muscle, but there is only limited information about dermatomyositis which might be of value to dermatologists.

N. R. ROWELL

Materials and Clothing in Health and Disease. E. T. RENBOURN. (1972) London: Lewis. Pp. 612, 51 illustrations. Price £9.50.

The origin of clothing is controversial. As a mantle of fur or hide it may have compensated for man's hairlessness and extended his territorial range when need for food drove him further from the protection of the forests. Or it may have originated as 'tropical dress', a decorative girdle on which to hang charms and ornaments. Or even, as with the natives of Southern Mindanao, merely as a utilitarian protection against leeches and spines—the thorn rather than the apple inspiring the fig-leaf. Indeed, modesty seems to have been a later reason for clothing but one which led imperceptibly to the function of dress as a sexually-directed form of adornment. This aspect has, of course, been well covered by Laver, Cunnington and others. So have the hygienic and protective aspects of clothing, especially in relation to industry, war and space exploration. The textile industry has also accumulated a great deal of experience about the physical and physiological attributes of natural and artificial clothing materials. But these different aspects of clothing have not until now been brought together in one volume. The author, a psychiatrist at Brookwood Hospital, has an exceptional background as a former lecturer in Applied Physiology and then Director of the Physiological Research Establishment of the Ministry of Defence. He is thus uniquely qualified for this task. Dr W. H. Rees, Principal Research Officer at the Cotton, Silk and Man-made Fibres Research Association, contributes a scholarly section on the Biophysics of Clothing Material. Other sections deal with the history and medical aspects of clothing, its biology, anatomy and physiology and those attributes of the body surface that are influenced by it—shape, thermal and climatic responses and environmental influences. The final section, on the psychology of dress, covers this wide field with wit, balance and commonsense. The work is summarized as 'an attempt to deal with clothing . . . as an aspect of human biology and social behaviour.'

The book is full of bizarre facts which will intrigue all these concerned with the vagaries of human behaviour and the history and customs of mankind. The Indians of Tierra del Fuego can sleep outside, even in snow, by wearing a piece of fur over one shoulder as a windbreak; copper wire woven into sock toes has been valued as an antifungal agent; saffron as a clothing dye is an antidote to lice; the flat noses of the Esquimaux protect them from frostbite.

The marks of illness shown by clothing are well-documented—the hat and glove size of the acromegalic, the diabetic shoe stain, carotid sinus syncope from tight collars, the 'pantie-girdle syndrome'; even 'clothes balls' in the mentally deranged. A compulsive washing neurosis leading to 'detergent dermatitis' is perhaps not sufficiently appreciated by dermatologists. It is salutary to read how physicians have decried each passing fashion in terms of the disease it was supposed to produce, e.g. tight lacing and chlorosis. Sometimes they were right—pointed shoes and corns were both common in the eighteenth century and Rigby, in 1785, first noted miliaria on the chests of over-swaddled infants.

In a work that ranges so widely it is not surprising that some specialized areas appear to be over-condensed or out-of-date. In dealing with the physiology of the skin, the author has not taken fully into account the considerable advances in barrier and water resevoir function during the last decade and his description of the method and significance of patch testing needs some revision. It is also surprising to read, in 1972, that 'scabies is an uncommon disease of man'. No doubt these few pages will be revised in the next edition.

The book is beautifully produced and the illustrations are apposite and well-chosen. Main references only are given for each section and, in a rather general manner, in the text. These might be usefully expanded in a work that deserves to be quoted as authoritative.

This fascinating and absorbing book should be bought, not borrowed. Whether as a reference book or as bedside reading, it is strongly recommended to every dermatologist who is interested in the inter-relationship of the patient, his skin and his environment.

D. S. WILKINSON

Symposium on Venereal Diseases. BRUCE WEBSTER (Ed.) (1972) The Medical Clinics of North America, Vol. 56, pp. 1055–1222. Philadelphia; Saunders, Price £3.40.

In Britain the treatment of venereal diseases is not in general regarded as coming within the province of the dermatologist. Of the many factors which have brought about this situation the most influential was the report of the Royal Commission on Venereal Disease in 1916; Sir Malcolm Morris, dermatologist to St Mary's Hospital, was a member. This report resulted in the establishment by local authorities of free clinics, most of which were not under the supervision of dermatologists. The advantages and disadvantages of splitting the traditional specialty of dermato-venereology have been much debated but the strong arguments marshalled by the protagonists on each side have lost their heat and few British dermatologists under the age of 50 now have experience in venereology. The entry of Britain into the EEC may however reopen the issue, for most dermatologists in the other countries in the Community do in fact treat venereal diseases, and the ultimate aim is reciprocity of specialist qualifications.

Whether or not the dermatologist in Britain treats venereal diseases he must be sufficiently familiar with their cutaneous manifestations to make a reliable diagnosis, for gonorrhoea is now one of the commonest infectious diseases and the frequent absence of genital symptoms in the female is bringing more late infections to departments of dermatology.

For rapid revision the new symposium edited by Bruce Webster is strongly recommended. The sixteen short chapters, with numerous references to the recent literature, provide concise authoritative reviews of the present state of knowledge. Five chapters are devoted to general aspects of venereal disease, four to gonorrhoea, three to syphilis and one each to herpes genitalis, non-specific urethritis and trichomonal infections. Of particular interest and importance to the dermatologist are the chapters on the complications of gonococcal infections by Kraus, on asymptomatic gonorrhoea by Pariser and on herpes genitalis by Young.

ARTHUR ROOK

Dermatologia. Jose Gomez Orbaneja (1972) Madrid: Aguilar. Pp. 689, 1244 black and white illustrations, 38 colour. Price £11.40.

Dr Gomez Orbaneja, the distinguished professor of dermatology in the University of Madrid, has set himself the difficult task of providing a comprehensive balanced text book for students and practitioners, a much more exacting task than either a brief introduction for students or a large reference book for specialists. As Dr Gomez writes in his prologue, the boundaries of any specialty are not easy to define and it follows that the decision as to what must be excluded and what admitted must depend on the author's assessment of the requirement of his readers. This book is an amplified version of the course of lectures given by Dr Gomez to his students; they are fortunate, for the fifty-three chapters cover lucidly and concisely the whole field of clinical dermatology and venereology. The book is beautifully produced and is a pleasure to handle and to read. The very numerous illustrations are informative and bear witness to the richness of the clinical material at the author's disposal.

Although no references to the literature are provided every chapter shows abundant evidence of the author's erudition, and the lack of the all too familiar distracting citations in the text certainly increases the reader's enjoyment. This book may be recommended to all dermatologists who can read Spanish without too painful an effort, for it summarizes the experience and opinions of a respected clinician whose range of clinical material differs in many respects from that encountered in Northwest Europe and North America.

The author was obviously obliged to make difficult decisions as to what should be excluded if the book was to remain of acceptable length. However, some omissions seem surprising. For example the jejunal lesions of dermatitis herpetiformis (here classified as benign pruritic pemphigus) are not discussed and the immunological aspects of the bullous eruptions are mentioned only very briefly. The newer work on the bacteriology of acne surely also deserves greater emphasis. Even so, the most serious criticism to be made of this otherwise useful book concerns the frequent lack of detailed advice on treatment. In the account of the treatment of acne there is not sufficient emphasis on antibiotics and no advice is given on the choice of antibiotic, the dosage or the duration of treatment. There is no mention of retinoic acid, although it is referred to elsewhere in the book. Less than half a page is given to the treatment of psoriasis and no detailed instructions are provided. In the management of rosacea appropriate diet, digestive enzymes, sedatives and antibiotics 'which may influence the intestinal flora', are recommended. A future edition will gain greatly if the sections on treatment are expanded and more detailed specific advice is given. Some procedures, the value of which has not been established by controlled trials, could be omitted.

Arthur Rook

Medicine in the Mining Industries. J. M. Rogan (Ed.) (1972) Heinemann: London. Pp. 397 + x, illustrations, some in colour. Price £4.50.

Judged by the number of pages and their content, this book will appeal mainly to the industrial physician interested in respiratory problems. The up-to-date information covers the common problems of pneumoconiosis and silicosis in coal mines of Britain, but extends also to mining of asbestos, uranium and other ores in other parts of the world. There are a number of concise chapters of help to management and mining engineers in respect of ergonomics, mining equipment, industrial hygiene, sickness absence and rehabilitation.

Those particularly interested in skin disorders will find the full list of common irritants and sensitizers which may cause contact dermatitis in British coal mines well documented. There is some information also of the very common 'wear and tear' eczema and about the management of tinea pedis and recommended first-aid treatment of cutaneous disorders.

Inclusions of special hazards of temperature and humidity, e.g. miliaria and non-dermatophyte fungal infections, and disorders likely to be found in other types of mines in the world, would have perhaps rounded off the excellent information available.

There is ample reward within the book for those who in the words of the author are 'concerned with the health of the miner'.

G. A. Hodgson

British Journal of Dermatology (1973) **88**, 313.

Quarterly Review

DRUG REACTIONS XXI

Cutaneous Reactions to Cardiovascular Drugs

DIURETICS

The thiazides

This group of diuretics includes chlorothiazide and hydrochlorothiazide. These two drugs are aromatic sulphonamide preparations with closely related formulae. Thus they may produce eczematous eruptions when administered systemically to patients sensitized by the topical use of sulphonamides. They have also been shown to be potent photosensitizers (Fisher, 1966). Jung & Schwarz (1965) demonstrated cross-sensitivity between 4-chloro-2-hydroxybenzoic acid-N-n-butylamide (Jadit) and various sulphonamide derivatives, including chlorothiazide and hydrochlorothiazide. Buckhardt & Sutter (1963) reported six patients with eczematous eruptions occurring on light exposed areas of skin due to hydrochlorothiazide. Photo-patch tests confirmed the sensitivity. Norins (1959) reported a non-pruritic petechial eruption occurring on the exposed areas of an 87-year-old woman. Harber, Lashinsky & Baer (1959) described three patients with photosensitive eruptions due to chlorothiazide and hydrochlorothiazide. Two of these patients showed the morphological and histopathological features of lichen planus, while the eruption in the third was vesicular and erythematous. The action spectrum in photo-sensitization due to these drugs was shown to be in the wave-length range between 275 nm and 310 nm, i.e. in the sunburn part of the ultraviolet spectrum. A lichenoid eruption may also occur unassociated with photo-sensitivity. Other reactions include an erythematous papular eruption sparing the trunk (Rogin, 1958), and vasculitis. Bjornberg & Gisslen (1965) found a history of previous medication with thiazides in a series of patients with necrotizing vasculitis. Using a provocative dose of hydrochlorothiazide they reproduced a severe vasculitis. When this vasculitis had subsided, a second provocative dose was given, this time with chlorthalidone. Relapse of the lesions occurred together with haematuria. Both thrombocytopenic purpura (Ball, 1960) and non-thrombocytopenic purpura of a Henoch-Schönlein type (Reid, Stevenson & Orr, 1969) have been reported. Chrein & Rubin (1962) described a patient with agranulocytosis secondary to hydrochlorothiazide therapy.

Frusemide

Frusemide is also a sulphonamide derivative, and is a more powerful diuretic than the thiazides. Cutaneous reactions to this drug are very rare. Erythema multiforme occurred in a 65-year-old man who was receiving frusemide (Gibson & Blue, 1970). No provocation test was performed. Ebringer, Adam & Parkin, (1969) described a 64-year-old man who developed haemorrhagic bullous lesions on his limbs after high doses of frusemide. The lesions disappeared on changing to a non-sulphonamide diuretic. Biopsy of a lesion revealed a subepidermal bulla. The patient was treated with prednisolone and the bullae did not reappear when it was stopped. The authors suggest that this eruption may have been the direct result of frusemide, although bullous pemphigold was offered as a differential diagnosis. As with the thiazides, cross-sensitivity has been demonstrated to 4-chloro-2-hydroxybenzoic acid-N-n-butylamide (Jung & Schwarz, 1965).

Ethacrynic acid

Ethacrynic acid is a powerful oral diuretic comparable in potency to frusemide. It is not a sulphonamide derivative and reported cutaneous eruptions are rare. A Henoch-Schönlein-like eruption was reported in two patients (Bar-On, Eisenburg & Eliakim, 1967). Necrotic haemorrhagic lesions developed without thrombocytopenia and histology showed a vasculitis. One other patient had been reported who developed acute gastric ulceration with widespread ecchymoses (Pain, 1967).

Mercurial diuretics

Organic mercurial diuretics are now rarely used because most have to be injected, and less toxic alternatives are available. The commonest allergic eruptions include scarlatiniform or morbilliform erythema and urticaria. Often these manifestations are accompanied by fever and malaise. If exposure is continued, a generalized exfoliative dermatitis may develop (Billo, 1941). A fixed eruption has been reported (Derbes, 1964).

Other diuretics

Spironolactone and triamterene, which are both potassium-sparing diuretics, have few cutaneous side-effects.

HYPOTENSIVE AGENTS

Hydralazine

Because of its various side effects hydralazine is now rarely prescribed. This drug was introduced in 1951 for the treatment of hypertension. 2 years later rheumatic and febrile reactions to hydralazine therapy resembling rheumatoid arthritis and systemic lupus erythematosus began to be described. The incidence of this reaction is between 8% and 13%. Alarcón-Segovia *et al.* (1967) extensively reviewed the literature and studied fifty patients with the syndrome. In thirty-seven patients the reactions followed administration of the drug for more than 6 months, while in the other thirteen patients such manifestations occurred acutely after less than 30 days of medication. The initial reactions in their patients consisted mostly of fever, high ESR, arthritis, leucopenia and skin rash. Several patients developed pleuro-pericardial disease and evidence of renal involvement. Thirteen patients had some cutaneous disturbance at the time of the hydralazine reaction. Ten patients had an erythematous rash. In one, the rash had the characteristic butterfly pattern. Another patient had erythematous plaques on the occipital region before treatment with hydralazine was begun; she developed a generalized papular eruption 10 days after hydralazine therapy was started. Another patient developed a macular rash on the face and arms as well as petechiae on the trunk, all of which disappeared within 2 days after withdrawal of hydralazine. Other cutaneous manifestations included two patients with exfoliative dermatitis, and two with livedo reticularis. Two patients developed photosensitivity to sunlight. All but two of the thirteen patients had demonstrable LE factor.

Reserpine

Cutaneous reactions are rare, but about 5% of patients develop nasal stuffiness, dry mouth or diarrhoea.

Methyl dopa

Methyl dopa is the alpha-methylated derivative of dihydroxy-phenylamine (DOPA). A common manifestation of sensitivity is drug fever, which may or not be accompanied by a rash (Tallgren & Servo, 1969). Few cutaneous reactions have been reported. Dollery (1965) described a number of patients who developed a pruritic papular rash mainly on the legs. The eruption cleared on stopping the drug. Peterkin & Kahn (1969) reported four patients who developed a keratotic eczema affecting the palms and soles. In two patients the eczema became more diffuse until the drug was stopped. A third patient resumed therapy and the eczema reappeared within 3 days. Generalized pruritis (Oates *et al.*, 1965) and thrombocytopenia (Benraad & Schoenaker, 1965) have both been recorded. Other rare manifestations include cheilitis (Mackie & Mackie, 1966), ulceration of the lower leg (Mackie, 1967), hyperpigmentation (Varadi & Lawrence, 1969) and a lichenoid eruption (Stevenson, 1971).

Guanethidine

Guanethidine is a post-ganglionic adrenergic-blocking drug. Cutaneous reactions are rare. Rastogi (1966) reported a fixed eruption. Dewar & Peaston (1964) described three cases in which 'collagen vascular disease' developed during treatment of long-standing hypertension with guanethidine.

Debrisoquine

Debrisoquine is an adrenergic blocking drug, acting at the neuro-affector junctions. Bauer (1970) studied thirty-four patients receiving the drug over a period of 5 years. One patient developed a maculo-papular rash which disappeared soon after the cessation of the drug. Talbot (1965) described a similar eruption in one patient. Other reports are rare and the types of rash have not been specified.

Clonidine

Clonidine is a recently introduced anti-hypertensive agent. It is an imidazoline derivative, chemically related to phenolamine and tolazoline. Its mode of action is uncertain. There is evidence of both a central and peripheral action. Skin reactions are infrequent and include maculo-papular eruptions and Raynaud's phenomenon (Catapres Symposium, 1969). After taking clonidine for 4 weeks, a 28-year-old hypertensive man presented with dry mouth, drowsiness and a dry scaly rash over the dorsal aspect of the hands with bulla formation and arthralgia. He also developed Raynaud's phenomenon. Symptoms cleared on cessation of therapy. No other cause could be found for the reaction and no LE cells were found.

ANTI-ARRHYTHMIC AND ANTI-ANGINAL AGENTS

Digitalis glycosides

Allergic reactions to digitalis are rare in spite of its widespread use for many years. The cutaneous manifestations include urticaria, scarlatiniform eruptions, papular and vesicular lesions, and oedema (Grettve & Johansson, 1958). Morrow (1887) published one of the first reviews of digitalis allergy. He also observed a generalized erythematous eruption in a patient, progressing to urticaria with intense pruritis, facial oedema and hyperpyrexia. The eruption disappeared after ceasing digitalis medication. Desquamation, complete alopecia of the scalp and shedding of the finger and toe nails ensued. Mosko & Taylor (1954) described a man who developed angioedema with bullae on his arms. A similar reaction occurred on subsequent exposures to any form of digitalis. Grettve & Johansson (1958) reported a 60-year-old woman who while taking digitalis leaf developed generalized pruritis and bullae on her legs. When her therapy was chaged to digitoxin, in addition to the previous lesions, she developed multiple oval light brown pigmented areas with an elevated erythematous edge and in some cases a red central papule. After withdrawal of digitoxin the eruptions disappeared, only to recur when acetyl digitoxin or lanatoside-C was substituted. The patient also had an eosinophilia, although isolated eosinophilia associated with digitalis therapy has been reported on several occasions (Smith & Beuner, 1931; Romano & Geiger, 1936). Wolfe & Geiger (1953) observed cross-sensitivity in an 82-year-old woman who developed urticaria on exposure to the digitalis glycosides. Highly purified as well as crude preparations were involved, and they suggested an allergic response attributable to the common cyclopentenophenanthrene nucleus. Cohen & Brodsky (1940) found that patch tests and intradermal tests, using tincture of digitalis and powdered leaf were negative in a patient who developed urticaria. Thrombocytopenic purpura is another rare manifestation of digitalis allergy. Berger (1952) reported a patient who developed reversible thrombocytopenia when taking digitoxin. When the drug was given again there was another fall in the platelet count.

Quinidine

Quinidine is the dextrorotatory isomer of quinine, and is used in prevention and control of cardiac arrhythmias. Fever, alone or accompanied by other symptoms, is a more frequent manifestation of hypersensitivity (Foley & Perada, 1966). Sturnick (1942) observed a patient in whom pyrexia occurred a few hours after each administration of an oral dose of quinidine. On each occasion fever was accompanied by leucopenia and transient splenomegaly. Cutaneous manifestations of quinidine sensitivity are uncommon. They include morbilliform and scarlatiniform eruptions, and urticaria (Shaftel & Halpern, 1958). Taylor & Potashnick (1951) reported a patient in whom a maculo-papular rash progressed into a severe exfoliative dermatitis. The exfoliation subsided rapidly when quinidine was withdrawn but recurred when the drug was readministered. Quinidine has also induced a severe erythrodermic exacerbation of psoriasis (Baker, 1966; Gourgerot, Bathélemy & Weill, 1930). Rarely, a fixed drug eruption may occur. Goldschlag (1942) reported a fixed eczematous eruption occurring in a 64-year-old man in whom, on a subsequent occasion, a generalized scarlatiniform rash developed. Lichenoid eruptions resulting from quinidine therapy were discussed fully in a previous quarterly review (Almeyda & Levantine, 1971). Such eruptions are uncommon but well recognized manifestations of quinidine sensitivity. Several patients with thrombocytopenic purpura following quinidine therapy have been reported (Taylor & Potashnick, 1951). Fernstrom (1965) reported a patient who developed a contact dermatitis following exposure to quinidine when grinding the sulphate in the pharmaceutical industry. He remarked that contact sensitivity to quinine is well recognized but that its occurrence with quinidine had not been reported previously.

Procainamide

Procainamide is useful in the prevention of ectopic beats and the control of ventricular arrhythmias. The production of a lupus erythematosus-like syndrome has become well recognized since it was first reported (Ladd, 1962). Baker & Moore-Robinson (1970) discussed the syndrome fully in a previous quarterly review. Rosin (1967) described a patient with vasculitis affecting the digits of his hands. Two lupus erythematous preparations were positive but no other manifestations of systemic lupus erythematosus were present. Cutaneous manifestations of procainamide sensitivity are otherwise very rare.

Propanolol

Propanolol is a beta-adrenergic-blocking drug. It is used in the treatment of angina, as an anti-hypertensive agent, and for the control of certain arrhythmias, such as those produced by digoxin toxicity or by thyrotoxicosis. Cutaneous side effects are infrequent, and are most commonly erythematous. Stephen (1966) described six patients who developed such rashes within 48 h of starting the drug. In five of these patients the rash cleared following cessation of the drug and in the sixth patient it disappeared while treatment was continued. Hebb, Godwin & Gunton (1968) reported cutaneous reactions occurring in five out of twenty-eight patients. These included flushing, perianal eruption, mild pruritus, a maculo-papular rash on the arms and face, and an acneiform eruption. After several months of therapy, propanolol had to be writhdrawn from one hyperthyroid patient because of the development of erythema multiforme (Pimstone *et al.*, 1969). Mild pruritus was also described by Humphreys & Delvin (1968) and rashes of an unspecified nature have been reported (Coté & Drance, 1968).

Practolol

Practolol is a more recently introduced beta-adrenergic-blocking agent which has similar uses to propanolol. Wiseman (1971) assessed the unwanted effects in over 2100 patients receiving practolol, and reported rashes in only seven patients. Two patients had maculo-papular eruptions, two were eczematous, one urticarial and two unspecified. Assam & Banks (1973) report a patient with a scaly erythematous eruption with LE cells present and positive antinuclear factor. They correlated this rash with practolol therapy and noted that no reaction occurred to propanolol which had been administered previously. Lymphocyte transformation was positive with practolol but not significant with propanolol. The rash and LE cells disappeared when practolol was discontinued.

Glyceryl trinitrate and pentaerythritol tetranitrate

Glyceryl trinitrate and pentaerythritol tetranitrate are vasodilator agents used in the treatment of angina pectoris. Cutaneous sensitivity to these drugs is uncommon and appears to occur most commonly with pentaerythritol tetranitrate (Goodman & Gilman, 1970). Ryan (1972) described a man who developed a bright red erythematous rash, with desquamation, most marked on the palms, soles and perineum. There was oedema affecting all the limbs. He had been taking pentaerythritol tetranitrate for 8 years. The eruption cleared 14 days after withdrawal of the drug. The rash recurred when the patient took two further tablets of glyceryl trinitrate, so demonstrating cross-sensitivity.

JOHN ALMEYDA
The Enfield Group of Hospitals
ASHLEY LEVANTINE
The Middlesex Hospital,
London, W.1

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News and Notices

The British Association of Dermatology

Applications are invited for the *DOWLING TRAVELLING FELLOWSHIP* which is open to registered practitioners who are British graduates and who hold a higher medical qualification. The aim of the Fellowship is to encourage evident merit in junior physicians working in dermatology, before they become established in senior academic and professional posts.

Candidates must have a suitable training and experience in clinical dermatology, in laboratory medicine and in research methods; they will normally hold an appointment in a dermatological unit or department of a Teaching Hospital in the United Kingdom. They are required to furnish an essay on the nature of the work to be carried out and indicate at what centres the necessary facilities could be provided.

The Fellowship, which is normally awarded every third year, will be tenable for one academic year and will be of value up to £2000. There is no bar to the successful Fellow seeking other monies to supplement this award if deemed necessary.

Enquiries or applications, which should be submitted before 1 January 1974, to be made to the Hon. Secretary, Dr J.S.Pegum, The London Hospital, Whitechapel Road, London E1 1BB.

University of Cambridge Postgraduate Medical School

The fourth of the Courses held at 5 yearly intervals and entitled 'Progress in the Biological Sciences in Relation to Dermatology' will take place in Cambridge from 24 to 29 September 1973. The Course is intended primarily for clinicians of considerable experience, but anyone who is interested is welcome to attend. Details of the programme and accommodation may be obtained from the Secretary of the Medical School, J.W.Woodcock, M.A., Hills Road, Cambridge.

14th Congress of the Association of French-speaking Dermatologists

The 14th Congress of the Association des Dermatologistes et Syphiligraphes de Langue Française is to be held in Geneva from 31 May to 3 June 1973. President: Professor P.Laugier, Secretary: Dr N. Hunziker.

The two main themes will be toxicoderma and allergic vasculitis.

Applications for registration should be made to the Secretary, Clinique dermatologique, Universitaire de Genève, Hôpital Cantonal—1211 Genève 4.

APPOINTMENTS

University of Bristol

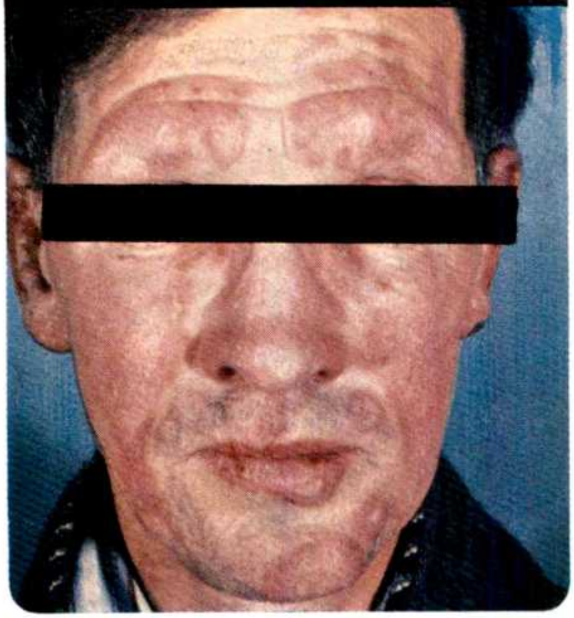
Dr J.L.Burton has been appointed Consultant Lecturer at the University of Bristol. He graduated M.B., Ch.B. (Manchester) in 1964 and became M.R.C.P. (London) in 1967. He held House Officer appointments at the Manchester Royal Infirmary, Hammersmith Hospital and Brompton Hospital and from 1966 to 1968 was Lecturer in Medicine at the Edinburgh Royal Infirmary. From 1968 to 1971 he was first Registrar and then Senior Registrar in the Department of Dermatology, Newcastle-upon-Tyne, where he is at present working as a Wellcome Research Fellow. He has published a considerable number of papers on clinical and investigative dermatology and general medicine. His main research interest is in the physiology and pathology of the sebaceous gland.

Lewisham Hospital, London

Dr Dorothy Isabella Vollum has been appointed Consultant Dermatologist to the Lewisham Hospital, London, England. She qualified M.B., B.S. (London) in 1960 and obtained her D.C.H. in 1964, having trained in paediatrics at the Radcliffe Infirmary, Oxford. She proceeded to take her M.R.C.P. in 1965. She became Senior House Officer at St John's Hospital for Diseases of the Skin, London and then Registrar and Senior Registrar at King's College Hospital. For a time she worked with Dr Gunnar Lomholt in the Department of Dermatology in the Mulago Hospital, Kampala, Uganda. She has published a number of papers on clinical and investigative dermatology.

A site more difficult to treat

This particularly resistant case of discoid lupus erythematosis called for an occlusive steroid dressing.
But conventional treatment would have been extremely difficult..



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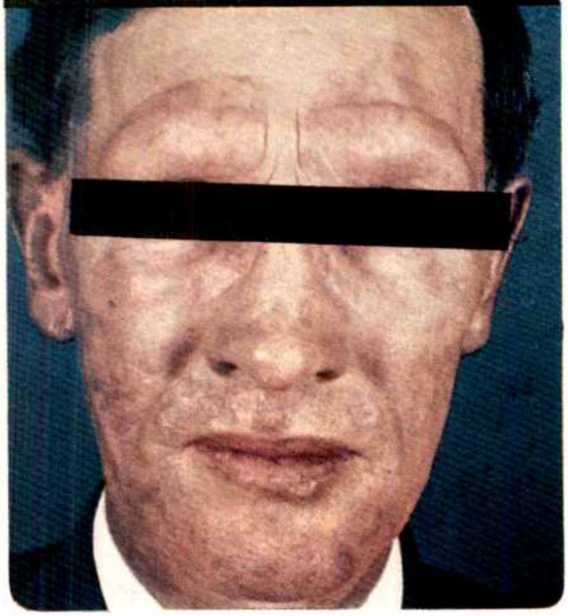

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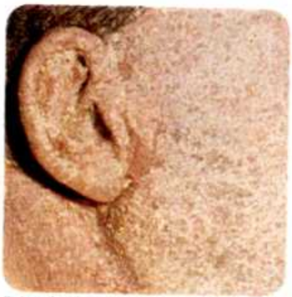
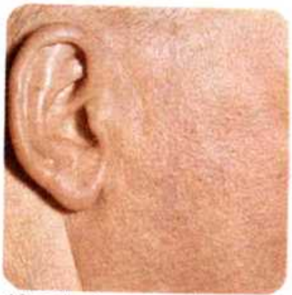
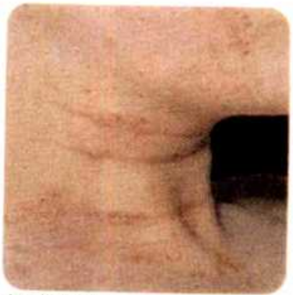
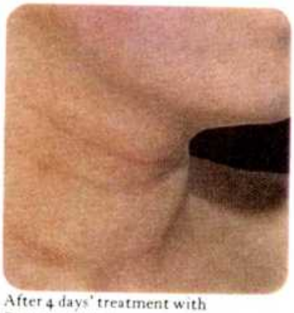
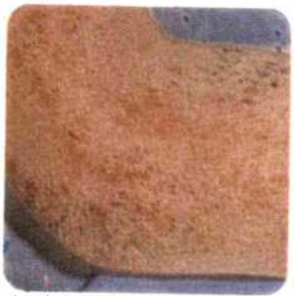
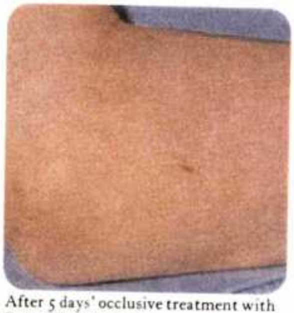

Seborrhoeic dermatitis since childhood

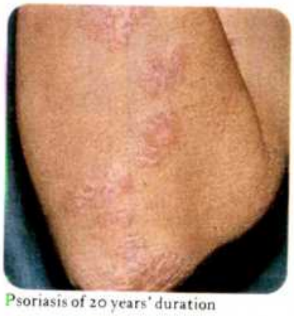

After 7 days' treatment with Betnovate Ointment

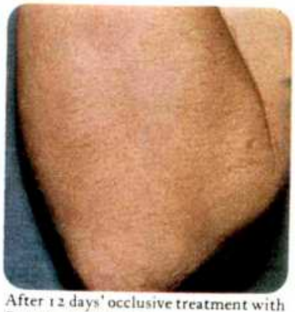

Atopic eczema

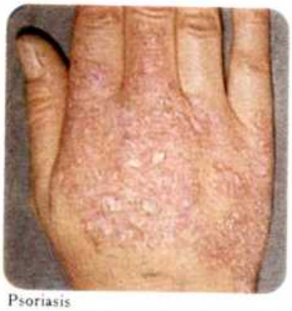

After 4 days' treatment with Betnovate Ointment

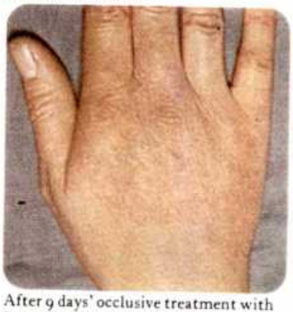

Atopic dermatitis of 3 years' duration

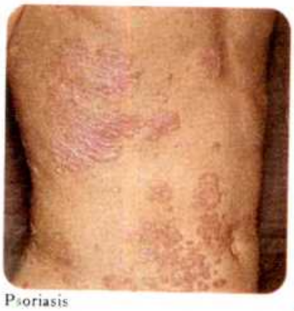

After 5 days' occlusive treatment with Betnovate Ointment

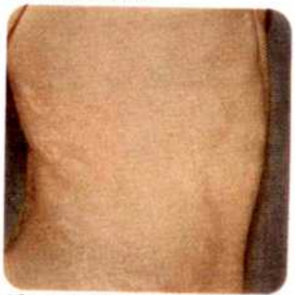

Psoriasis of 20 years' duration

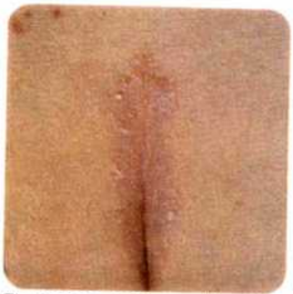

After 12 days' occlusive treatment with Betnovate Ointment

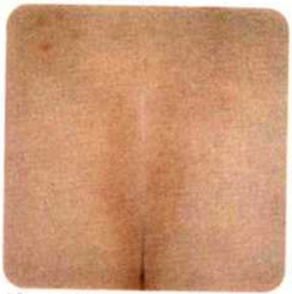

Psoriasis

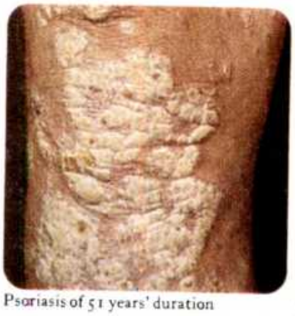

After 9 days' occlusive treatment with Betnovate Ointment

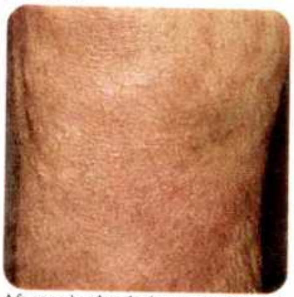

Psoriasis


After 21 days' occlusive treatment with Betnovate


Flexural psoriasis of 15 years' duration

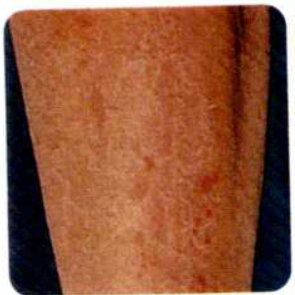

After 4 weeks' treatment with Betnovate Ointment

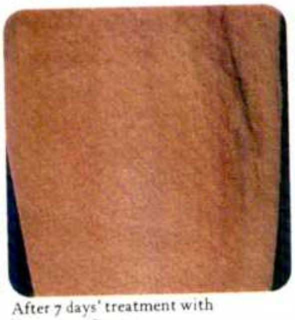

Psoriasis of 51 years' duration

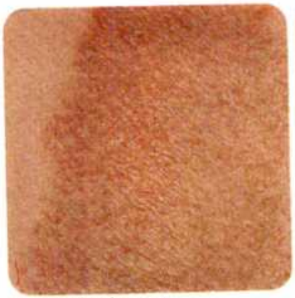

After 24 days' occlusive treatment with Betnovate Ointment

the results.

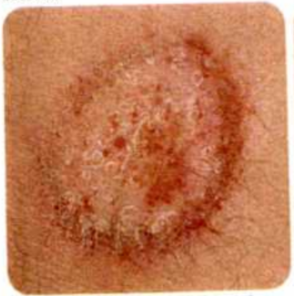
again. And again.

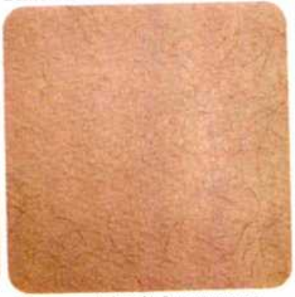

Varicose eczema of 4/5 months' duration

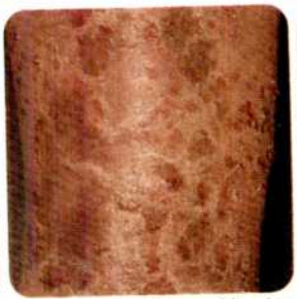

After 7 days' treatment with Betnovate Cream

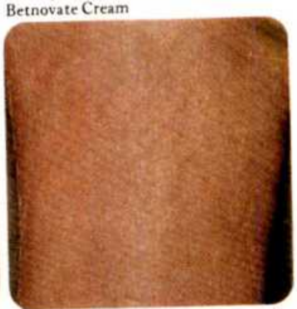

Constitutional eczema

After 4 weeks' treatment with Betnovate Cream


Discoid eczema of 9 months' duration


After 2 weeks' occlusive treatment with Betnovate Ointment


Atopic dermatitis of 12 years' duration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> "...a better response is more likely with betamethasone 17-valerate."*

*Lancet (1964) 1, 1,177

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15, 30 and 100 gram tubes
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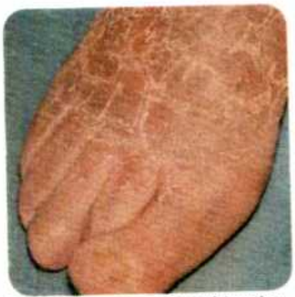
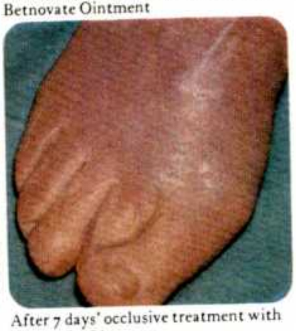

Contact dermatitis of 2 years' duration


After 7 days' occlusive treatment with Betnovate Ointment

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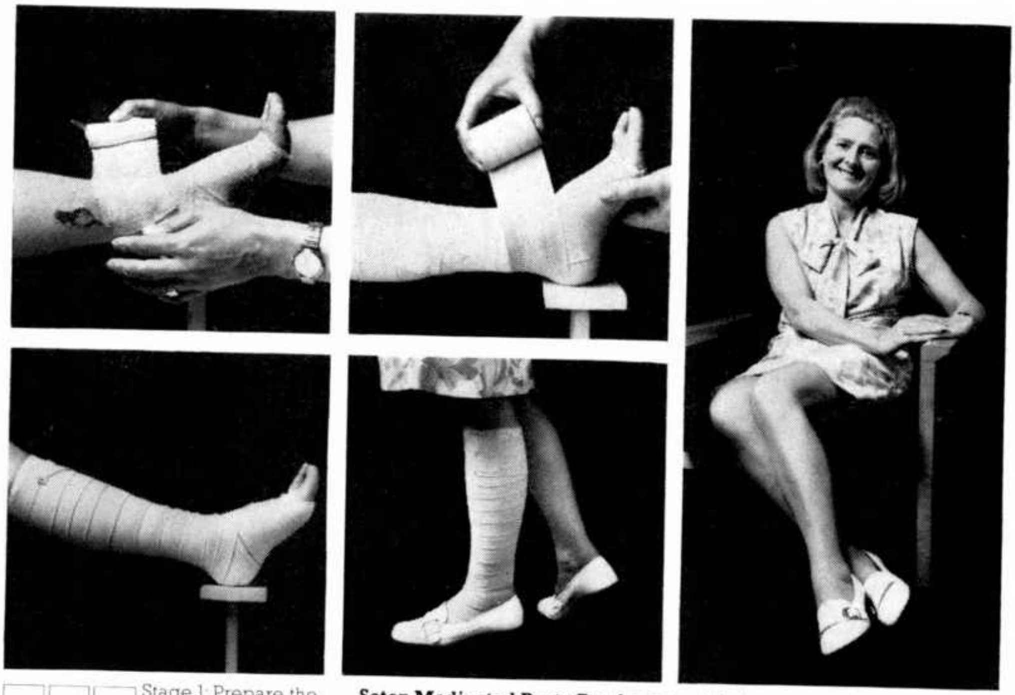
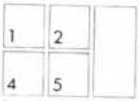
The effective treatment of leg ulcers...by Seton





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Tarband (Zinc Paste and Coal Tar/BPC)
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elastic diachylon bandages, plain or ventilated.
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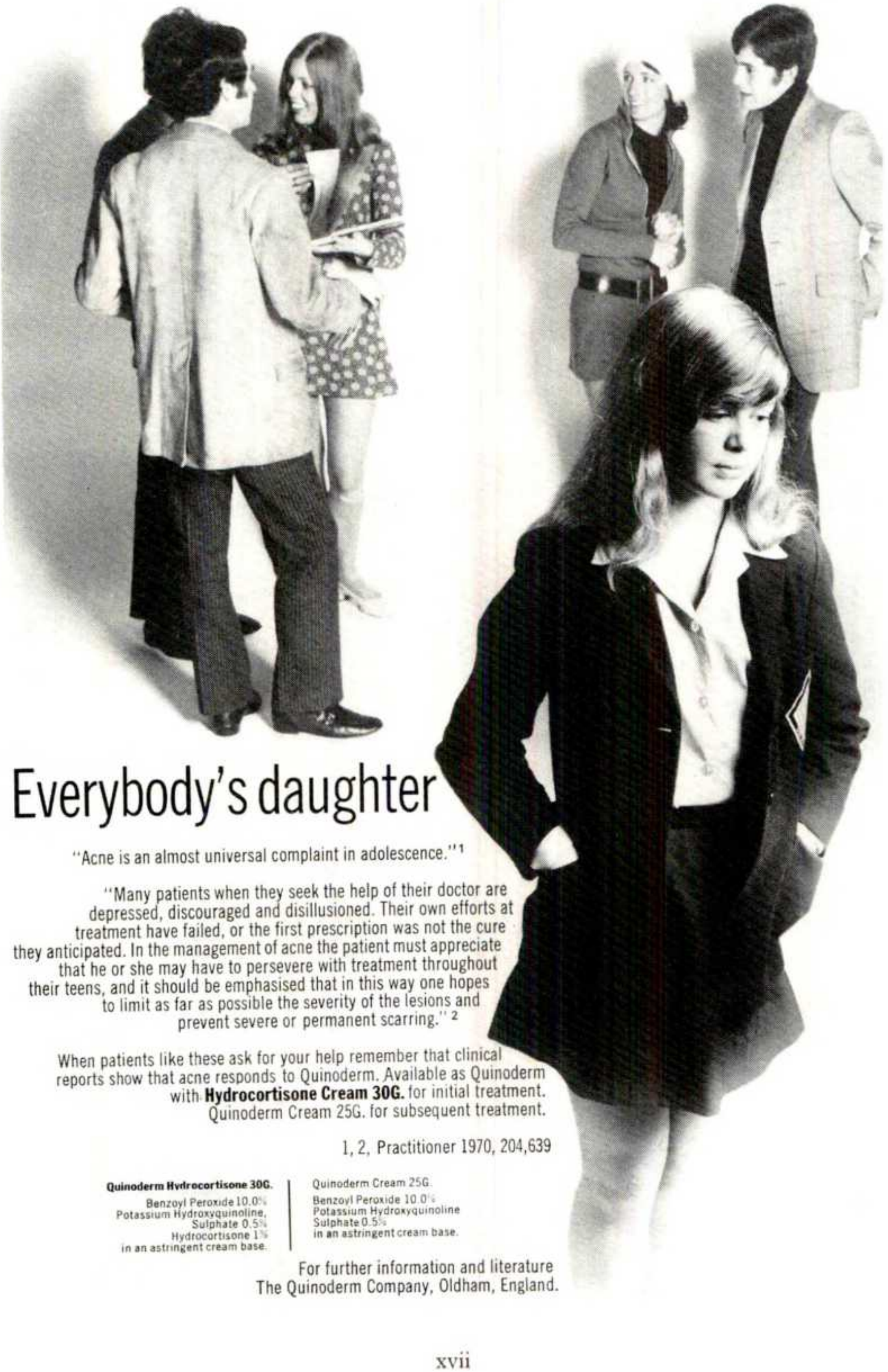

Everybody's daughter

"Acne is an almost universal complaint in adolescence."[1]

"Many patients when they seek the help of their doctor are depressed, discouraged and disillusioned. Their own efforts at treatment have failed, or the first prescription was not the cure they anticipated. In the management of acne the patient must appreciate that he or she may have to persevere with treatment throughout their teens, and it should be emphasised that in this way one hopes to limit as far as possible the severity of the lesions and prevent severe or permanent scarring."[2]

When patients like these ask for your help remember that clinical reports show that acne responds to Quinoderm. Available as Quinoderm with **Hydrocortisone Cream 30G.** for initial treatment. Quinoderm Cream 25G. for subsequent treatment.

1, 2, Practitioner 1970, 204,639

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A simple once-a-night treatment – safe in pregnancy, free from side effects and providing rapid relief of symptoms.

2½% Suspension the treatment of choice for broncho-pulmonary infections caused by candida and aspergillus species. Also used to treat oral thrush, particularly in the neonatus.

Pimafucin 2% Cream is recommended for use in fungal skin infections, particularly those caused by candida, microspora and tricophyton species. Also for the treatment of candidal balanitis, oral thrush and candidal infections associated with ill-fitting dentures (has no unpleasant taste). Frequently used as an adjunct to Pimafucin Vaginal Tablets in treating vulvo-vaginitis, for more immediate relief of severe pruritis.

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Journal of the Society of

Cosmetic Chemists

of Great Britain

Edited by J. M. Blakeway

Vol. 24 No. 4 April 1973

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Phyllis C. Flawn, S. A. Malcolm and R. C. S. Woodroffe. Assessment of the preservative capacity of shampoos.

J. D. Middleton and B. M. Allen. The influence of temperature and humidity on stratum corneum and its relation to skin chapping.

T. A. Bakiewicz. A critical evaluation of the methods available for measurement of antiperspirancy.

N. Senior. Some observations on the formulation and properties of chlorhexidine.

The *Journal of the Society of Cosmetic Chemists* is published thirteen times a year at £21.00 ($50.40) per annum, post free. New subscriptions and requests for specimen copies of the journal should be addressed to Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL.

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Dermatologica

Offizielles Organ der Schweizerischen Gesellschaft für Dermatologie und Venerologie
Organe officiel de la Société Suisse de Dermatologie et de Vénérologie
Official Organ of the Nederlandse Vereniging van Dermatologen

Editors: *J. Delacrétaz* (Lausanne); *W. Jadassohn* (Geneva); *L.H. Jansen* (Utrecht); *M.K. Polano* (Leiden), and *R. Schuppli* (Basel)

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Matheis, H. (Basel): Amiodarone-Pigmentierung
(Amiodarone Pigmentation)

Brun, F.; Sellei, K. und *Krebs, A.* (Bern): ATP-ase-Bestimmungen im menschlichen Spermaplasma
(Measurement of ATP-ase-Activity in Human Seminal Plasma)

Zala, L. (Bern): Histologische Befunde bei Behandlung von Hautneoplasien mit 5-Fluorouracil-Salbe
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Textbook of Dermatology

Edited by

Arthur Rook M.D. F.R.C.P., D.S. Wilkinson M.D. F.R.C.P. and
F.J.G. Ebling D.SC. PH.D.

The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this edition is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

List of Contributors

Harvey Baker M.D.(Leeds) M.R.C.P.(Lond.)
John Martin Beare M.D.(Belfast) F.R.C.P.(Lond.)
Robert John Cairns M.B. B.S.(Lond.) F.R.C.P.(Lond.)
Robert Harold Champion M.A. M.B. B.CHIR.(Cantab.) M.R.C.P.(Lond.)
Francis John Govier Ebling D.SC. PH.D.(Bristol)
Sigfrid Fregert M.D.(Lund)
James Clark Gentles B.SC. PH.D.(Glasgow)
Roger Richard Martin Harman M.B. B.S.(Lond.) F.R.C.P.(Lond.)
Niels Hjorth M.D.(Copenhagen)
William Henry Jopling F.R.C.P.(Edin.) F.R.C.P.(Lond.) D.T.M.&H.(Eng.)
Francisco Kerdel-Vagas M.D.(Caracas) M.SC.(N.Y.)
Gunnar Lomholt M.D.(Copenhagen)
Donald William Ross Mackenzie B.SC. PH.D.
Jack Nagington M.D.(Manchester)
William Everett Parish M.A. PH.D.(Cantab.) B.V.SC.(Liv.) M.R.C.V.S. M.R.C.PATH.
Jens Jørgen Pindborg D.D.S. DR.ODONT.
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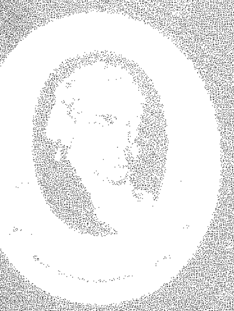

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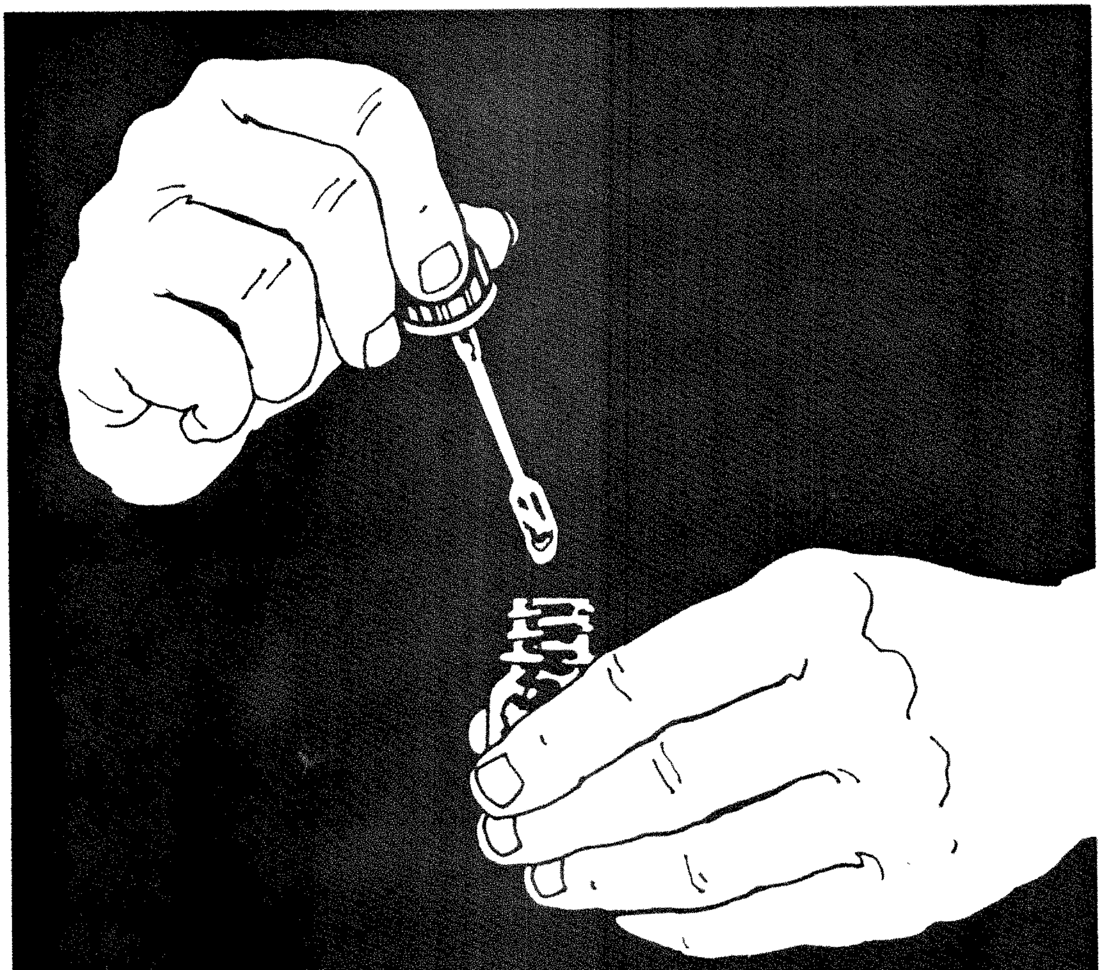

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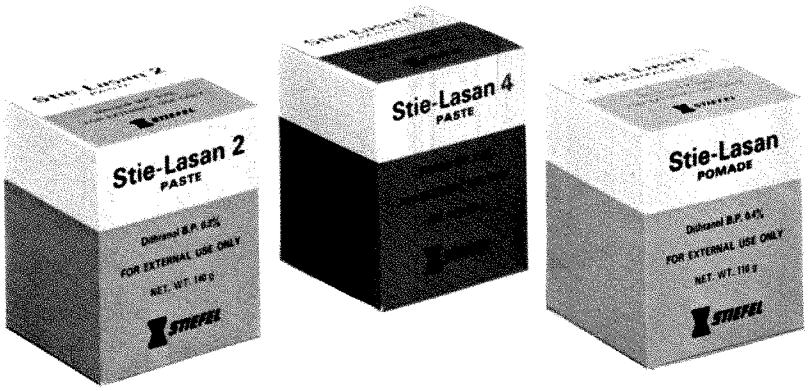

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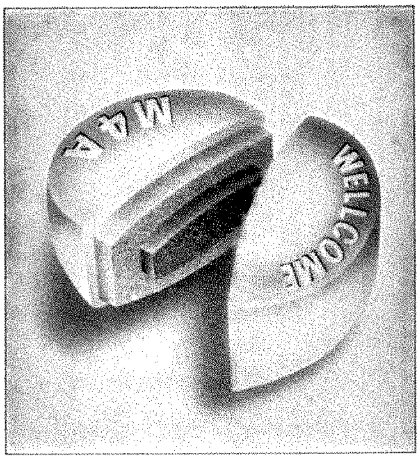

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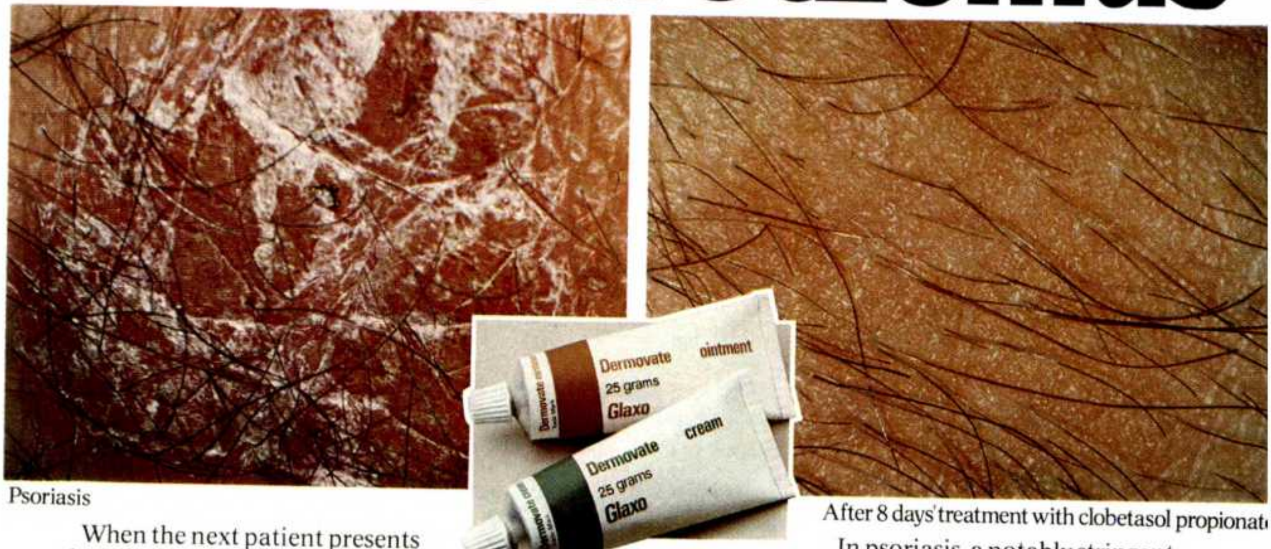

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Clinical and Laboratory Investigations

Ultrastructural observations on uninvolved skin in dermatitis herpetiformis

D.J.RICHES, B.G.H.MARTIN, P.P.SEAH AND LIONEL FRY

Departments of Anatomy and Dermatology,
St Mary's Hospital Medical School, London, W.2

Accepted for publication 25 September 1972

SUMMARY

Clinically normal skin from patients being treated for dermatitis herpetiformis was examined with the electron microscope.

Cytoplasmic processes from the basal epidermal cells penetrated the dermis through discontinuities in the basal lamina which otherwise was normal in structure and thickness. The fibrous network of the dermis consisting of collagen, small reticular fibrils, anchoring fibrils and elastin, appeared normal.

In patients receiving only dapsone, membrane-bound 'vacuoles' were found just below the basal lamina. They contained a fibrillar material of low electron density and were often associated with cell processes or complete cells, the classification of which was difficult. It is suggested that these vacuoles may be implicated in the reaction between reticulin and immunological complexes.

In two specimens, early blister formation was also seen in the dermal papillae. The basal lamina remained applied to the stratum basale and was only absent where basal cell processes projected into the blister space which contained fibrin deposits. Vesiculated structures observed in the region of the blisters are interpreted as abnormal sensory nerve endings.

This evidence supports the view that disruption of the basal lamina and blister formation in dermatitis herpetiformis are secondary to an earlier reaction.

Light microscope observations have established that in dermatitis herpetiformis, the skin lesions are situated at or below the dermal–epidermal junction of the dermal papillae (Pierard & Whimster, 1961). In the formation of the typical blister characterizing these lesions, the epidermis is separated from the dermis and examination with the electron microscope has revealed that in the region of the separation the basal lamina is disrupted or absent (Bellone & Caputo, 1966; Pierard & Kint, 1968; Fry & Johnson, 1969; Jakubowicz, Dabrowski & Maciejewski, 1970; Rodrigo, 1972); from this evidence it has been suggested that the basal lamina is primarily involved with the disease process (Fry & Johnson, 1969; Jakubowicz *et al.*, 1970). Recent investigations on clinically normal skin in dermatitis herpetiformis using immunological techniques have shown that antireticulin antibodies are present beneath, but probably not involving, the basal lamina of the dermal papillae (Seah *et al.*, 1972). Therefore, it was felt necessary to examine the dermal–epidermal junction of uninvolved skin in dermatitis herpetiformis at the ultrastructural level. This paper describes changes which have been observed in the dermis of clinically normal skin from patients with dermatitis herpetiformis.

MATERIALS AND METHODS

Specimens were taken from a series of twelve patients with dermatitis herpetiformis in whom the rash was being treated with dapsone and/or a gluten-free diet. Five patients were on dapsone alone, three were solely on a gluten-free diet and four were on both dapsone and a gluten-free diet. All skin areas chosen were clinically normal. Eleven biopsies were taken from the gluteal region and one from the forearm; they were performed under 2% lignocaine anaesthesia. The tissue was fixed in 4% buffered glutaraldehyde and immediately post-fixed in 1% buffered osmium tetroxide. After dehydration with a graded series of ethyl alcohols, the specimens were embedded in Taab resin. Thick sections, stained with toluidine blue, were examined to aid orientation of the tissue; subsequently thin sections were cut, stained with uranyl acetate and/or lead citrate (Reynolds, 1963) and examined on a Siemens Elmiskop I electron microscope.

RESULTS

In general, the structure of the basal cells of the epidermis was normal, but in some specimens cytoplasmic processes from the dermal surface of basal cells were seen passing through discontinuities in the basal lamina to penetrate the dermis for short distances (Fig. 1). These processes were usually smaller than those described in the region of a blister (Fry & Johnson, 1969), but they were similar

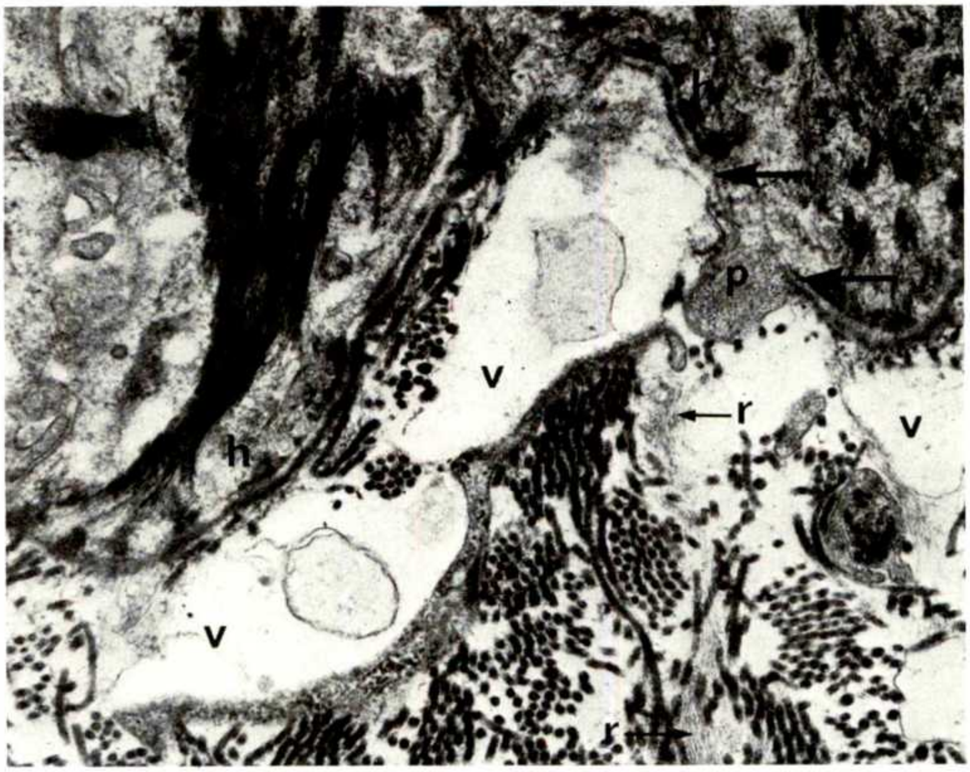

FIGURE 1. The dermal–epidermal junction showing vacuoles (v) in the dermis close to a basal cell process (p) which projects through a discontinuity in the basal lamina (thick arrows). Note the normal hemidesmosomes (h) and reticular fibrils (r) (× 20,800).

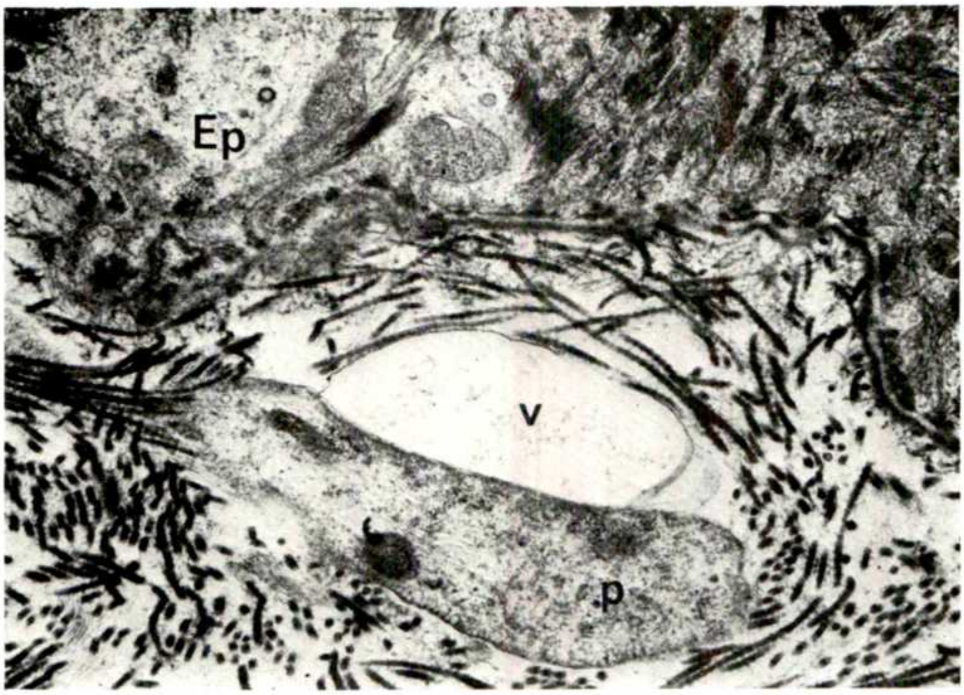

FIGURE 2. A membrane-bound vacuole (v) filled with a fibrillar material and associated with a cell process (p). Ep, epidermis (× 20,000).

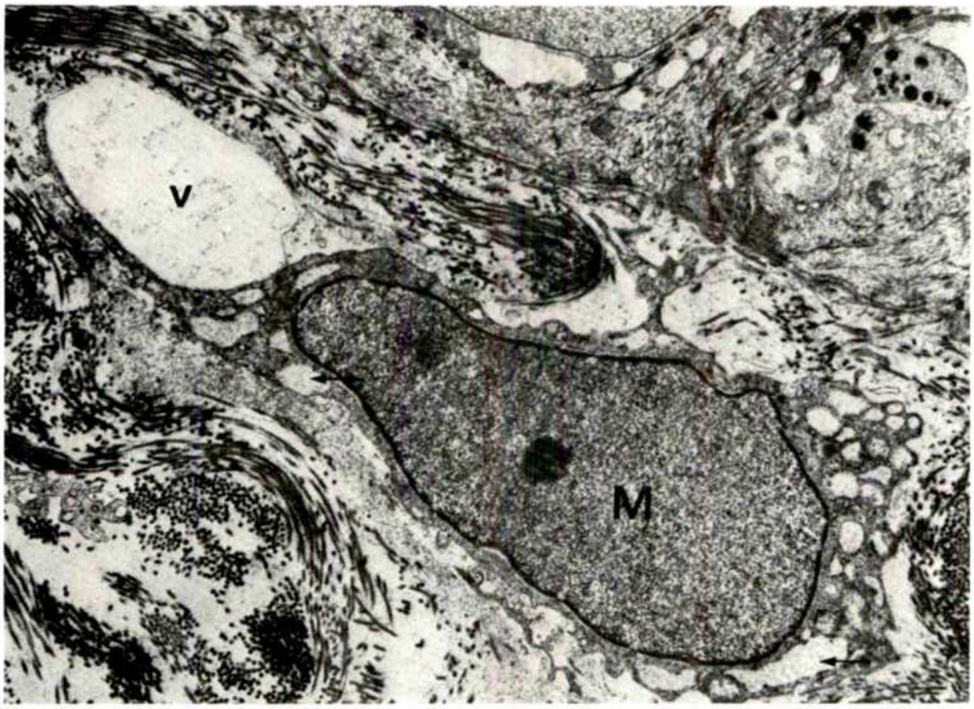

FIGURE 3. A vacuole (v) partly surrounded by a process from a dermal cell (M) which contains numerous vesicles (arrows) (× 7500).

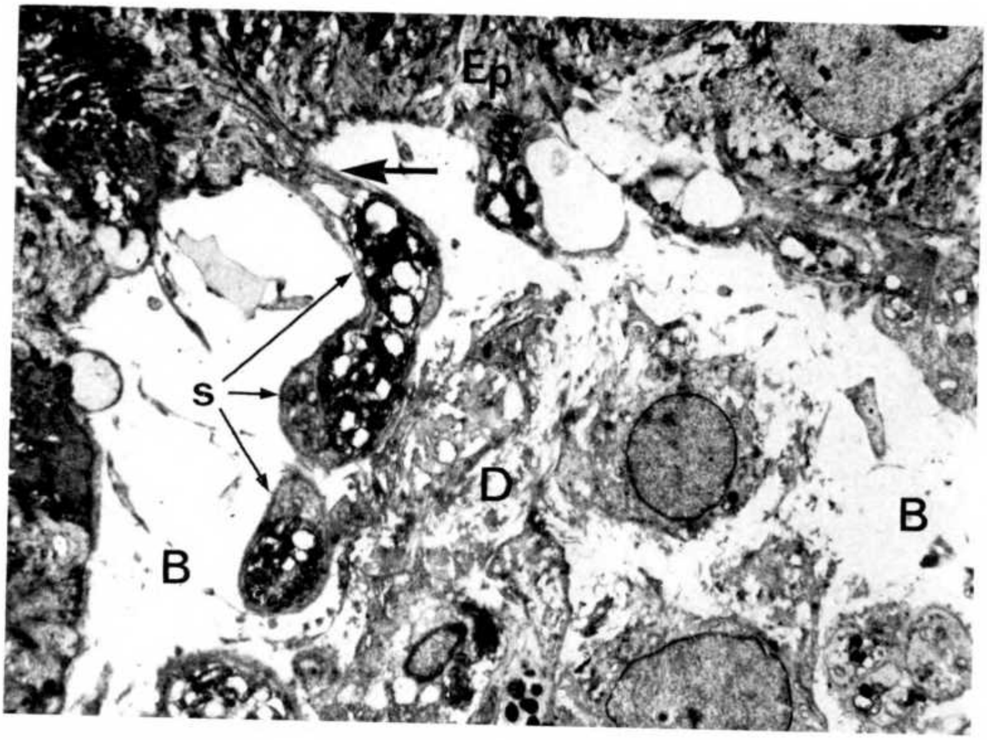

FIGURE 4. Early blister formation with separation of the epidermis (Ep) from the dermis (D) to produce a blister space (B). Note the vesiculated structures (s), one of which is associated with a basal cell process (thick arrow) (× 4000).

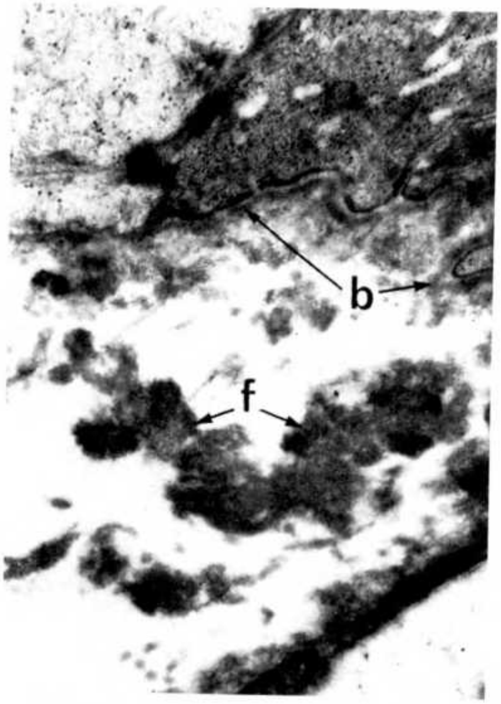

FIGURE 5. Fibrin deposits (f) close to the epidermal basal lamina (b) which is intact (× 20,000).

in that they did not contain cell organelles or tonofilaments. There was a normal arrangement of hemidesmosomes along the cell membrane bordering the dermis where the basal lamina was present (Fig. 1). The cytoplasm of the basal cells contained normal amounts of tonofilaments and the mitochondria and other cell organelles were normal in structure and distribution. Small deposits of glycogen were sometimes present.

The basal lamina appeared normal both in thickness and structure and, apart from occasional gaps where the basal cell processes penetrated the dermis (Fig. 1), it formed a continuous layer between the epidermis and dermis. Anchoring fibrils were present along its dermal surface.

The fibrous network of the dermis consisted of numerous collagen bundles interspersed by clumps of elastin; both these components were normal in appearance and distribution. Among the collagen fibres and subjacent to the basal lamina, groups of smaller 'reticular' fibrils were seen running for variable distances through the dermis (Fig. 1). Occasionally these fibrils formed clumps either close to the basal lamina or lying deeper in the dermis.

In all of the specimens taken from patients who were solely on dapsone therapy and in one from a patient on dapsone and a gluten-free diet, vacuole-like spaces were seen in the dermis just below the epidermal basal lamina and associated anchoring fibrils (Figs. 1–3). They were usually situated in the dermal papillae and in many cases they were found in the vicinity of the basal cell processes projecting into the dermis (Fig. 1). The 'vacuoles' were surrounded by a membrane which, in places, became indistinct and they contained a fibrillar material of low electron density. Sometimes a cell process (Fig. 2) or a complete cell surrounded part of the vacuole (Fig. 3); in all cases the membrane bounding the vacuole remained separate from that of the cell. In many respects these cells did not appear to be particularly well differentiated and consequently they were difficult to classify. Most of them had branching cell processes while in some cells the cytoplasm contained vesicles in which there was a material of low electron density (Fig. 3).

Specimens from two patients solely on dapsone had in addition to the vacuoles, evidence of early blister formation in some of the dermal papillae. In the region of such an early blister, most of the dermis was separated from the epidermis (Fig. 4), leaving the basal lamina and groups of fibrils still applied to the dermal surface of the stratum basale. The basal lamina was absent where processes from the basal epidermal cells projected into the blister space; these processes were longer than those which were observed in relation to the vacuoles. No actual thickening of the basal lamina was seen, but subjacent to it and within the blister space deposits of material resembling fibrin were present (Fig. 5). In the dermis beneath the blister space, there were several vesiculated structures (Figs. 4 and 6) and these were surrounded by cell processes, around the outer parts of which a basal lamina could be distinguished (Fig. 6). Some of the vesicles contained membranes which resembled the cristae of mitochondria (Fig. 6). Vesiculated structures were seen in contact with dermal processes of the basal cells (Fig. 4), but it was not possible to demonstrate the membranes clearly enough to observe the exact nature of the junction. Among the dermal elements beneath the blister, Schwann cell processes with unmyelinated nerve axons were also present.

Other components of the dermis were relatively normal. Macrophages and leukocytes were occasionally found, but rarely eosinophils. The capillaries showed no evidence of abnormality either in size or structure.

DISCUSSION

Previous ultrastructural studies on dermatitis herpetiformis have concentrated on the blister stage of the lesion when it is difficult to distinguish between the primary effects of the disease process and the non-specific secondary reaction. Rodrigo (1972) included in his material specimens of uninvolved

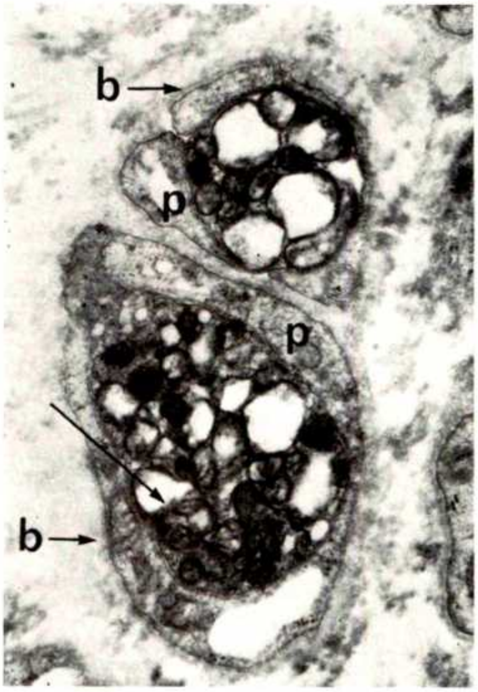

FIGURE 6. A higher magnification of two vesiculated structures which consist of a cell process (p) surrounded by a basal lamina (b) and enclosing numerous vesicles some of which have membranes resembling the cristae of mitochondria (arrow) (× 15,000).

skin from patients with blisters, but states that abnormalities in its structure were slight and inconstant. In the early blister, most investigators report that the basal lamina is disrupted or absent (Bellone & Caputo, 1966; Pierard & Kint, 1968; Fry & Johnson, 1969; Jakubowicz *et al.*, 1970; Rodrigo, 1972) and a fibrin-like material is deposited in the blister space (Jakubowicz *et al.*, 1970, 1971; Rodrigo, 1972). Although all the specimens in the present study were taken from clinically normal skin, two showed evidence of blister formation at the microscopic level. In these cases, the basal lamina appeared normal apart from breaks through which basal cell processes penetrated the dermis and the hemidesmosomes present did not exhibit the rarefaction which has been described in early lesions (Rodrigo, 1972). While the deposition of fibrin may be a characteristic of dermatitis herpetiformis (Jakubowicz *et al.*, 1971; Mustakallio, Blomqvist & Laiho, 1970), it is felt that this is probably secondary to an earlier reaction. Recent work on the experimental production of blisters has shown that a coagulum of fibrin and remains of basal lamina form a surface over which the regrowth of epidermal cells can occur (Krawczyk, 1971).

The vesiculated structures observed beneath the early blisters resemble in some respects the profiles described as sensory nerve endings (Burnstock & Iwayama, 1971) where the axon is packed with small, oval mitochondria. If the vesicles represent mitochondria, they are swollen and their cristae are disrupted. The presence of such apparently abnormal nerve endings in relation to the early lesion is interesting in view of the pruritus which is associated with the clinical pre-blister stage of the disease.

The fibrous network of the dermis contained in addition to collagen fibres, numerous smaller fibrils which correspond to the 'reticular' fibrils described in normal skin (Berger & Hundeiker, 1967). Whether these fibrils correspond to the tissue which is described as reticulin at the light microscope level has yet to be demonstrated, but they may be the structures onto which the 'antireticulin' antibodies described by Seah *et al.* (1972) are attached. There are certainly a number of such fibrils close to the basal lamina in the region where the antibodies are demonstrated.

The most obvious abnormal finding in clinically normal skin from patients solely on dapsone therapy was the presence of membrane-bound vacuoles in the dermis. The nature of the fibrillar material they contain cannot be resolved on the present evidence, but it could represent a collection of fluid induced by a local concentration of protein such as antibodies. Other possibilities include collections of ground substance or even cytoplasmic processes from epidermal cells similar to those seen in healing skin (Odland & Ross, 1968). However, the latter suggestion is unlikely as the vacuoles do not resemble the basal cell processes observed in the present investigation. Confluence of several vacuoles could well be the initial stage in the formation of the microscopic blisters previously discussed. Except for one specimen, the vacuoles were not seen in patients who were on a gluten-free diet with or without dapsone which raises the possibility that the presence of gluten in the diet of patients solely on dapsone therapy may still allow the pathological process to continue at a sub-clinical level. A more detailed study of the present findings in relation to treatment is in progress.

Owing to the difficulty in cell classification, the exact relationship of the dermal cells and the cell processes to the vacuoles could not be determined. The most probable explanation, however, is that the cells are some type of macrophage which are attempting to phagocytose the content of the vacuole. Likewise, the association between the dermal processes of the basal cells and the vacuoles is difficult to explain. Defects in the basal lamina, with dermal processes from basal cells, are rarely encountered in skin from normal adults (Riches, personal observations), but they have been observed in other skin diseases (Sugár, 1968; Cox, 1969). Cox (1969) suggests that basal lamina defects are in some way associated with cellular proliferation in the epidermis. Although this may explain the findings in psoriasis and other proliferative disorders, it is difficult to apply such an hypothesis to dermatitis herpetiformis where the cell turnover is probably normal. An alternative reason for dermal processes from basal cells in dermatitis herpetiformis could be that the basal cells are in some way secondarily implicated in the pathological process.

The findings of the present study are compatible with the results of Seah *et al.* (1972) who suggest that the primary site of the pathology in dermatitis herpetiformis is the reticulin fibres of the dermis which cross-react with immunological complexes. The changes in the basal lamina could be secondary to this reaction, and possibly due to local mechanical disruption; while the development of the blister with deposition of fibrin is consistent with an immune-complex disease.

ACKNOWLEDGMENT

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A case of bullous pemphigoid and figurate erythema in association with metastatic spread of carcinoma

N.K. SAIKIA, RONA M. MacKIE AND A. McQUEEN

University Department of Dermatology, Western Infirmary, Glasgow

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SUMMARY

Clinical and laboratory findings in a case of bullous pemphigoid developing during the course of metastatic spread of rectal adenocarcinoma are presented. In addition a transient figurate erythema occurred. The possibility of a causal relationship between bullous pemphigoid and malignant disease is discussed.

The possibility of a causal relationship existing between bullous dermatoses and neoplasia has been a subject of controversy since 1909 when the association was first postulated by Bogrow. Pemphigoid and carcinoma have frequently been reported in the same patient. Although Lim, MacDonald & Rook (1968) in their series of 103 cases of pemphigoid found no significant evidence that the two conditions were more than fortuitiously related, Hellier (1960), Wilson (1960) and Gold (1961), however, state that there is a causal association, particularly in cases where the buccal mucosa is severely affected.

In view of these contrasting viewpoints, and of the recent reports of the association of pemphigus with neoplasia (Saikia, 1972) and of pemphigus foliaceus with neoplasia (Saikia & MacConnell, 1972) we report here a further case of bullous pemphigoid, proven by strongly positive direct and indirect immunofluorescence, which was associated with rapid metastatic spread of adenocarcinoma of the colon.

CASE REPORT

W.K., a 48-year-old male crane-driver was admitted to the dermatological wards on 22 May 1971 for investigation and treatment of a bullous eruption of his trunk and limbs which had been present for 2 weeks prior to admission. The lesions were seen to arise on clinically normal skin, and were extremely itchy. There were no oral lesions at this time, and there was no past history of dermatological disorder.

Examination at admission showed large tense bullae present on the abdomen, upper arms and thighs. The bullae varied in size up to 5 cm in diameter, and in some cases rupture of the lesions had occurred, leaving denuded areas of skin.

In November 1970 the patient had undergone anterior resection of the rectum and partial cystectomy for an adenocarcinoma of the pelvirectal junction with associated colovesical fistula. His general health in the intervening period had been good, and he had gained 1·9 kg in weight. There were no symptoms suggestive of a recurrence of malignancy at this time, and general physical examination showed no abnormality.

Skin biopsy of an abdominal lesion was carried out, and the presence of small subepidermal bullae revealed. In addition there were many eosinophils present, both in the bullae and in perivascular areas within the dermis.

Differentiation between bullous pemphigoid and bullous erythema multiforme was not considered possible on the basis of study of the histological sections and therefore direct and indirect immunofluorescence studies were carried out. The direct test was performed with antihuman IgG, IgM, and IgA conjugate (Jordon *et al.*, 1967; Cormane & Szabo 1970). With IgG, conjugate basement zone immunofluorescence, typical of pemphigoid, was observed. The indirect test was carried out using the patient's serum in serial dilutions with fresh human vaginal tissue as substrate (Beutner *et al.*, 1965; Beutner, Jordon & Chorzelski, 1968). Basement zone immunofluorescence typical of pemphigoid was seen at antibody titres of 1 in 80.

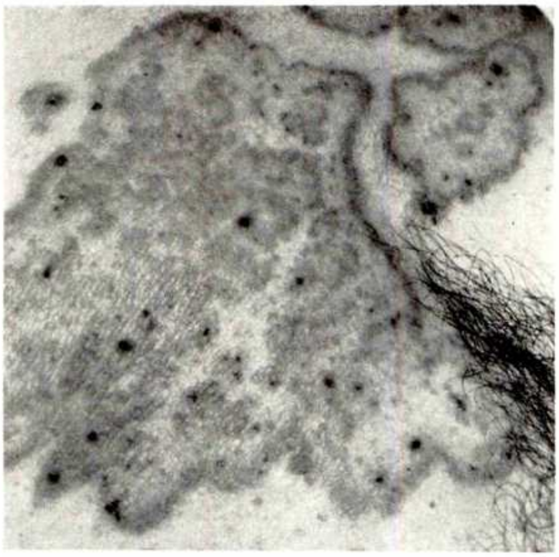

FIGURE 1. Figurate erythema which developed 6 weeks after onset of bullous eruption.

Other investigations carried out at this time, including full blood examination, electrolytes, urea, and liver function tests were within normal limits.

Treatment was started with oral prednisolone, the initial dose being 50 mg daily. This was reduced over the next 2 weeks to 5 mg daily. At this level fresh blisters appeared, and sulphapyridine was substituted in an attempt to wean the patient off steroids. This was unsuccessful as fresh lesions continued to appear until sulphapyridine was discontinued and prednisolone re-introduced at a dose of 10 mg daily. At this point in time lesions were first observed on the patient's buccal mucous membrane, and these persisted until his death.

On 20 June the patient developed colicky lower abdominal pain, with tenderness and guarding over the left iliac fossa. A subacute intestinal obstruction was diagnosed and this responded initially to an olive oil enema, but recurred 1 week later. Barium enema carried out at this time showed a narrowed segment near the rectosigmoid junction, presumed to be the site of the anastomosis. No evidence of recurrence of the neoplasm was observed. Hepatomegaly which persisted was noted at the time of the first subacute obstruction, but ultrasonic liver scan revealed no focal abnormalities suggestive of metastatic deposits. Serum alkaline phosphatase level was slightly raised at 16 KA units, and indirect immunofluorescence again showed basal zone immunofluorescence typical of pemphigoid, on this occasion at an antibody titre of 1 in 160.

For the next 3 weeks the patient continued to have episodes of intermittent abdominal colic, associated on two occasions with elevations of temperature. Throughout this period his bullous lesions persisted, despite a maintenance dose of 10 mg of prednisolone daily, and in addition he developed on the right side of his trunk a figurate erythema, the outline of which altered over the next 48 h, after which time it cleared spontaneously.

A technetium liver scan was carried out on 16 July and extensive loss of uptake was observed; the pattern was considered to be strongly suggestive of intrahepatic metastases. Surgical treatment for relief of his obstructive symptoms was therefore not contemplated and his abdominal pain was treated with analgesics. He died on 23 July and post-mortem examination was not carried out.

DISCUSSION

The association of bullous pemphigoid with metastatic spread of adenocarcinoma of the colon would appear in this case to be more than merely fortituous. A reason commonly postulated for the coincidental finding of these two conditions is the fact that both conditions occur in the elderly, but this patient was considerably younger than the average age for presentation of pemphigoid, 80% of patients first presenting over the age of 60 years (Sneddon & Church, 1955). In addition, the initial brief improvement of his dermatological condition, followed by deterioration and involvement of the oral mucosa, despite systemic steroid therapy coincided with the very rapid spread of his malignancy. The coincidental appearance of oral lesions and metastatic spread would lend support to the view that the appearance of such lesions should lead to a search for an underlying neoplasm in pemphigoid. This association has recently been discussed by Parsons & Savin (1968) in their series of seven cases of pemphigoid found in association with malignancy, three of which were found to have lesions on the mucous membranes. An additional interesting point made in this paper is the lack of correlation between the progress of the neoplastic condition and the bullous lesions, since in two cases the bullae cleared completely as the primary malignancy proceeded to secondary spread. This is in direct contrast to the case presented here in which the appearance of bullae coincided with metastatic spread of the malignancy, and no dermatological abnormality was observed at the time of presentation of the primary neoplastic lesion.

The brief presence of a figurate erythema is also worthy of note. Although localized to the right upper quadrant of the abdomen, this lesion had the woodgrain appearance as is seen in erythema gyratum repens in which 50% of recorded cases are associated with malignancy (Pevny, 1966; Thomson & Stankler, 1970).

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Skin lesions in tyrosinosis: response to dietary treatment

WITOLD A. ZALESKI, ALAN HILL AND WILLIAM KUSHNIRUK

Alvin Buckwold Centre, Department of Pediatrics,
University of Saskatchewan, Saskatoon, Saskatchewan, Canada

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SUMMARY

A chronic form of tyrosinosis was diagnosed in a mentally retarded girl, aged 13. Concomitant findings were skin lesions of localized epidermolytic hyperkeratosis existing without remission from infancy. A diet low in phenylalanine and tyrosine was started to reduce the patient's blood tyrosine to a normal level. After 6 months of successful dietary control of blood tyrosine, an unexpected result was a complete remission of the skin lesions. The diet was abandoned after a total period of 14 months and the abnormality of tyrosine metabolism became immediately prominent again. Some 4 months later, the skin lesions have recurred.

A possible relationship between the congenital skin disorder and the abnormal tyrosine metabolism in this case is considered.

It is well known that some inborn errors of amino acid metabolism may be associated with skin lesions.

The most common of the amino-acidaemias, phenylketonuria (PKU) is characteristically associated with decreased pigmentation. Infantile eczema, dry skin and non-specific rashes are also frequent occurrences and sensitivity to sunlight may be a problem. In some adult phenylketonuric subjects, eczema may persist in spite of topical treatment and, in our experience, such cases occasionally respond to a modified PKU diet and the lesions may heal in 4–5 weeks.

In Hartnup disease, a pellagra-like skin rash aggravated by sunlight is typically associated with periods of ataxia, mental retardation and characteristic amino-aciduria. Skin signs in homocystinuria are mild, consisting of livedo reticularis, a malar flush and light-coloured hair and skin.

In some cases of tyrosinosis, mild hyperpigmentation has been noted.

We wish to report an unusual skin lesion occurring concomitantly with elevated blood tyrosine and excretion of tyrosine and its derivatives in urine (tyrosyluria) in a mentally retarded girl diagnosed as a chronic form of tyrosinosis (Zaleski & Hill, 1973).

PATIENT AND METHODS

The patient was a girl aged 13 who was discovered on routine screening for inborn errors of metabolism of the residents at the provincial institution for the mentally retarded. Quantitative measurements of successive blood specimens, using an amino acid analyser, showed plasma tyrosine to be 25–30 mg/100 ml (normal <2·5 mg/100 ml).

Examination of the patient showed normal physical development and the only positive findings were nystagmus, strabismus and a chronic skin disorder. Extensive laboratory investigations, other than those mentioned above, were within normal limits. The patient was mentally grossly subnormal and had a severe behaviour disorder.

The patient's history indicated an uneventful pregnancy, birth and neonatal period. Corneal ulcers of obscure etiology appeared in infancy and persisted until the child was 4 years old. When the infant was 8 months old, the family noted 'blisters' on the flexor aspects of her fingers and on her soles. The family history revealed consanguinity and a case of psoriasis in one of the grandparents but otherwise was negative for cutaneous disorders. In spite of continuous topical treatment, the skin lesions became gradually worse, with the appearance of deep fissures and suppuration. With only minor variations, these changes persisted until the time of our investigation. At times the lesions were more than half an inch thick and caused such discomfort that the patient was unable to walk, but crawled on her knees. Eventually, the management of this child at home became impossible and at the age of 9 years she was admitted to an institution.

At that time, her skin lesions were described as clusters of linear, firm, hyperkeratotic, verrucous papules localized to the palms, the flexor aspects of the fingers, the soles and the flexor aspects of the toes, and surrounded by erythema and desquamating hyperkeratosis. Fissures were prominent over the joints.

Skin biopsy

A skin biopsy of a well-developed plantar lesion revealed changes of epidermolytic hyperkeratosis with epidermal acanthosis and a thick hyperkeratotic papillomatous surface. There was a bulla in the upper epidermis with a reticulated pattern in the underlying stratum spinosum associated with intercellular and intracellular oedema. The bulla was covered by thick hyperkeratosis and parakeratosis (Fig. 1). The cells of the thickened granular layer were large, contained coarse granules and exhibited perinuclear halos. Cells in the base of the bulla were eosinophilic and had perinuclear vacuolization (Fig. 2). There were occasional areas of individual cell keratinization and multinucleated cells in the epidermis. The bulla contained red blood cells, bacteria and leucocytes from secondary infection. The PAS stain indicated an intact basement membrane. The dermal papillae were oedematous with a perivascular infiltrate. An earlier palmar lesion presented similar histological changes with no evidence of infection.

Results of treatment

A diet, with restricted tyrosine–phenylalanine intake and supplementary amino acids, was started with the object of reducing the blood tyrosine to a normal level (Hill, Nordin & Zaleski, 1970).

We were successful in stabilizing the blood tyrosine at approximately 5 mg/100 ml, and after 6 months of treatment the lesions on both hands and feet had completely disappeared and the skin looked quite healthy except for some glossiness and a very mild hyperaemia. This improvement in the skin was maintained for a further 8 months during which the patient continued the phenylalanine–tyrosine-restricted diet. The patient was very active and quite comfortable throughout this period; no new local skin treatment was given.

As the diet was considered only experimental, it was abandoned after a total period of 14 months.

After approximately 4 months on a regular diet, the skin condition on the palms and soles began to deteriorate. After 6 months, marked hyperkeratotic areas reappeared and are spreading and thickening gradually. The patient is receiving topical applications and vitamin supplements, without much effect.

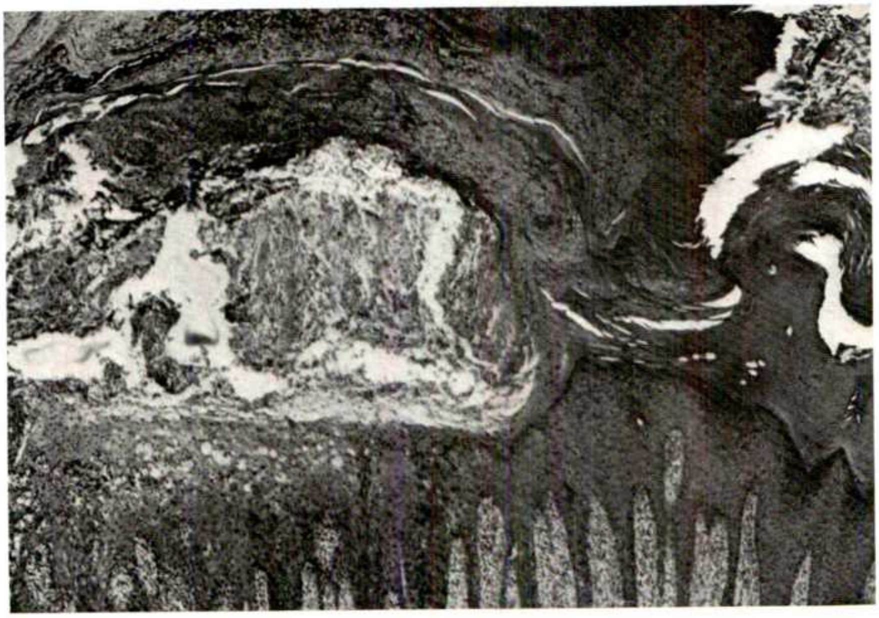

FIGURE 1. Biopsy of plantar lesion showing hyperkeratotic papillomatous surface, bulla, prominent granular layer and vacuolar changes in the epidermis (H & E, ×13).

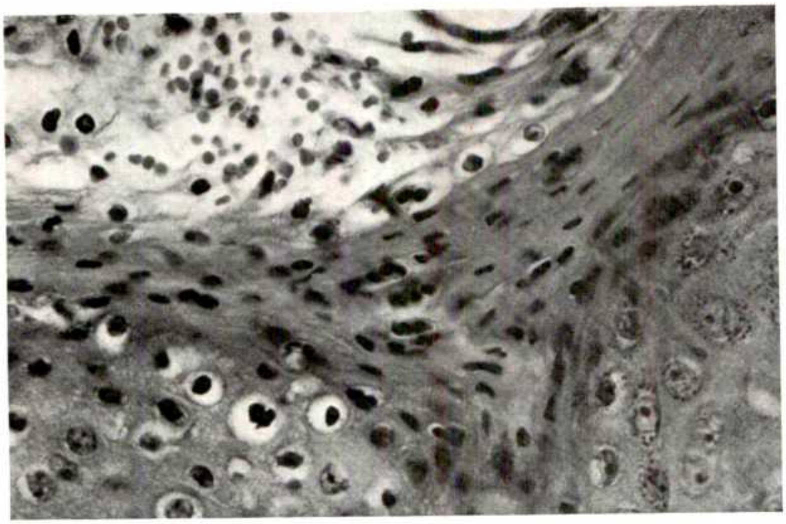

FIGURE 2. Higher magnification of bulla base, perinuclear spaces in the stratum spinosum and stratum granulosum with increased basophilic keratohyalin-like bodies (H & E, ×128).

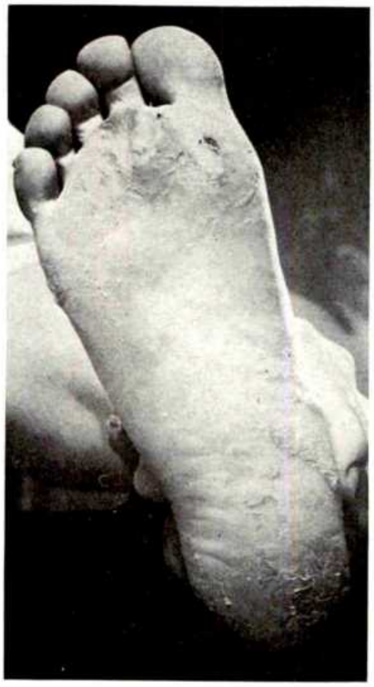

FIGURE 3. Recurrence of skin lesions on feet 6 months after discontinuation of phenylalanine-tyrosine restricted diet. Similar changes were present on both soles.

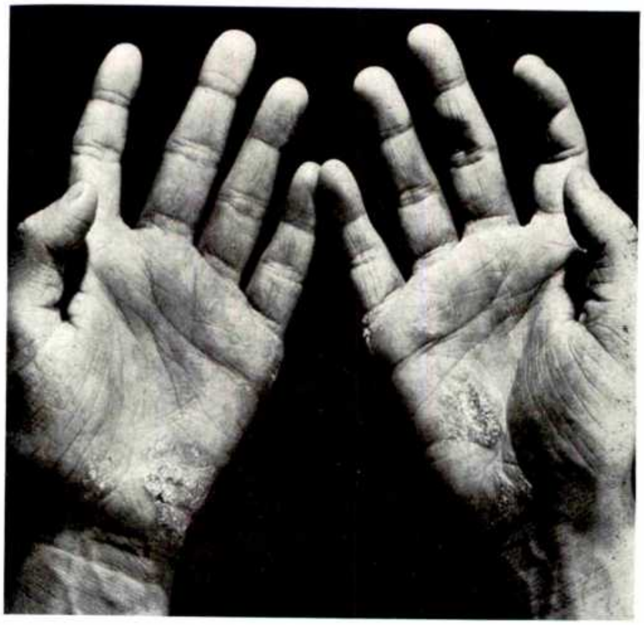

FIGURE 4. Recurrence of skin lesions on hands 6 months after discontinuation of phenylalanine–tyrosine restricted diet.

During the most recent dermatological examination, the skin was lichenified on the knuckles. Linear clusters of slightly raised hyperkeratotic lesions were found on the medial aspects of the heels and on pressure areas of the soles (Fig. 3). Linear hyperkeratotic papules were present on the hypothenar eminences, with extension to the little fingers (Fig. 4). There were no buccal or nail changes.

DISCUSSION

The clinical and histological features of this patient's lesions classify her condition as localized naevoid epidermolytic hyperkeratosis.

Ebling & Rook (1968) refer to circumscribed palmo-plantar keratoderma with these histological changes and state that this rare form may be a feature of more than one genetically distinct syndrome but that detailed case reports are too few to allow any firm conclusion. It is mentioned that these lesions may be associated with mental retardation and corneal dystrophies, as in our patient.

Reed, Galvanek & Lubritz (1964) described four cases of bullous congenital ichthyosiform hyperkeratosis. They surveyed similar cases in the literature and refer to the localized forms of this disorder. Mental retardation was noted in one of the children reported by the authors as well as in several previously reported cases. However, there is no indication that investigations for abnormal amino acid metabolism were done in any of the cases.

Frost & Van Scott (1966) discuss both the generalized and localized forms of ichthyosiform dermatoses, showing similar histological pictures, but again there is no indication of measurement of amino acid levels in blood or urine.

In an electron microscopic study of epidermolytic hyperkeratosis, Wilgram & Caulfield (1965) suggest that there are morphological signs of increased metabolic activity in the epidermal cells.

In his discussion of the inherited ichthyoses, Schnyder (1970) suggests that in epidermolytic hyperkeratosis, the most essential changes revealed by electron microscopy consist of faulty and increased formation of tonofibrils in the prickle cells. This author stresses that 'biochemical analyses should be the next step in the research of ichthyoses'. One might consider whether, in certain circumstances, the over-abundance of an important substrate such as tyrosine might selectively stimulate cellular metabolism in the epidermis.

In its most frequent form, tyrosinosis presents clinically as an acute progressive illness with signs of liver and kidney failure appearing in infancy. The biochemical manifestations include high plasma tyrosine and increased quantities of tyrosine and its metabolites in urine (Hill & Zaleski, 1971). The clinically mild variant with high plasma tyrosine is rare and only three other cases in the literature closely resemble our patient (Wadman *et al.*, 1968; Holston *et al.*, 1971; Buist, Campbell & Koler, 1969).

Kogut, Shaw & Donnell (1967) describe a case of tyrosinosis with liver and kidney failure where, during the course of dietary treatment, the patient developed a severe, desquamating rash. This was considered to be due to vitamin deficiency and responded to biotin therapy. The rash was similar to those seen in cases of phenylketonuria and galactosaemia on a synthetic diet.

Three possible explanations for the improvement in the chronic dermatological anomaly associated with tyrosinosis in our patient should be considered. The first possibility is that any apparent relationship of the skin condition to the abnormal tyrosine metabolism was fortuitous. The second explanation, which we favour, assumes a relationship of the skin lesions to the abnormal tyrosine metabolism. Although such an association has not been observed in other cases of tyrosinosis, the variant reported here may involve unknown factors which are responsible for the different phenotypical manifestations. It is possible that a subclinical naevoid skin condition may be aggravated by the raised blood tyrosine. The third possibility which might be considered is that there is no relationship of the patient's skin

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lesion to the abnormal tyrosine metabolism and that the improvement after several months of dietary treatment occurred, because of some supplement which the patient received in the protein hydrolyzate used to provide her amino acid requirements. The product used contains all the essential amino acids, minerals and vitamins, but only minimal quantities of phenylalanine and tyrosine (experimental product '3200 AB' provided through the courtesy of Mead Johnson (Canada) Ltd, Toronto).

Finally, it would be interesting to investigate whether undetected abnormalities in tyrosine metabolism might be present in association with other poorly understood congenital dermatoses which frequently defy diagnosis and treatment.

ACKNOWLEDGMENT

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Atypical lichen planus induced by native medicine

FEMI SOYINKA*

Faculty of Health Sciences, University of Ife, Ile-Ife, Nigeria

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SUMMARY

Two cases of bullous and nodular lichen planus induced by ingested and topically administered native medicine are described. The high incidence of lichen planus in Africa could be due to the widespread use of herbal drugs. This could partly explain the absence of buccal mucosal lesion in lichen planus in African Negroes.

Lichen planus (LP) is common in Africa; it accounts for 5% of new consultations in Senegal (Privat & Faye, 1967), 6% in Nigeria (Harman, 1962), and for 1–15% of our own cases, depending upon the type of people and their environment. In contrast, the incidence of new cases of lichen planus between 1952 and 1965 at St John's Hospital, London was 1·2% (Neves, 1966).

No attempt was made by earlier workers to distinguish between true lichen planus and lichenoid drug eruptions in the cases of LP reported from Africa. Most of the drugs and chemicals mentioned in the literature (Graciansky *et al.*, 1958; Woods, 1968; Pegum, 1968; Almeyda & Levantine, 1971) as capable of producing lichenoid eruptions are also widely used in Africa. Furthermore, native medicine still competes strongly with modern medicine throughout Africa. The herbs employed possess pharmacological and toxic properties which could cause specific side-effects. They are used without regard for dosage (it is usually said 'the more the better'), so that the incidence of reactions may well be high. Lichenoid drug eruptions which are clinically and histologically not easily distinguishable from true lichen planus (Sarkany *et al.*, 1958), probably account for part of the reported incidence of LP in Africa.

In his editorial comment on the paper of Zawahry on Lichen Planus Tropicus, Marshall (1969) observed: 'There are yet other curiosities about lichen planus in tropical Africa. In West and Central African Negroes the skin lesions tend to be more florid and exuberant than they are in Caucasians, and lesions of the buccal mucosa are rare in all African Negroes'.

The aim of this paper is to draw attention to the factors in LP in Africa that may be responsible for its high incidence and that may make the LP eruption in Africans different from that in other countries, and to attempt to explain why lesions on the buccal mucosa are rare in most African Negroes.

CASE HISTORIES

Case 1

A woman aged 49 was admitted with an extensive rash which she said had started more than 2 months before. The lesions had begun as blisters on the breast, with intense pruritus. A few days later,

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* Present address: Hadassah Medical School, Jerusalem.

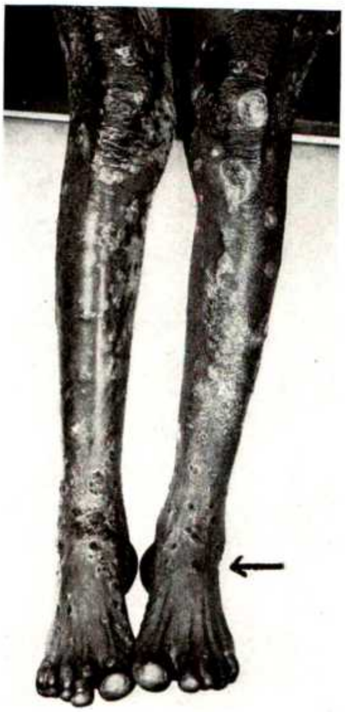

FIGURE 1. Hypertrophic lichen planus of the legs (Case 1). Note especially the darkly pigmented nodules on the dorsum of left foot.

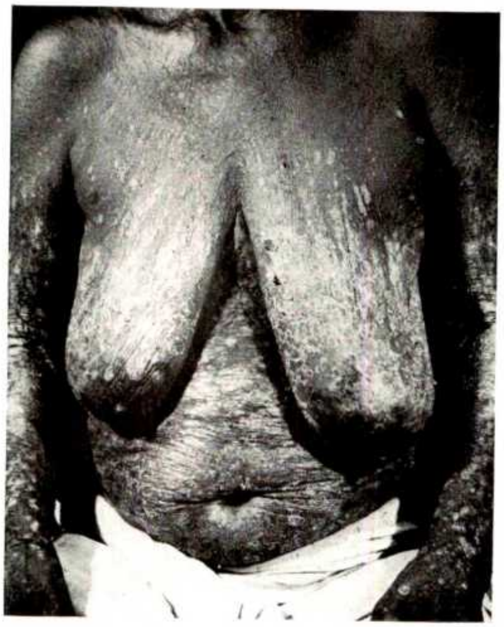

FIGURE 2. Bullous lichen planus (Case 1) in the healing phase.

papular lesions appeared on the breast, trunk, and legs and became hypertrophic after 2 weeks. Past medical history was unremarkable.

There was an extensive lichen planus-like eruption sparing the face, scalp, and hands. The lesions on the legs were remarkably hypertrophic. There were nodules on the legs about 2 × 2·5 cm, very firm, and darkly pigmented. Their surface was rough, whitish, and had a crater-like centre about 0·5 cm in diameter, resembling an umbilicated lesion of molluscum cantagiosum (Fig. 1). The bullous eruptions were widespread on the breast and chest. Typical lichen planus lesions on the trunk were scanty (Fig. 2). No lesion of the buccal mucosae was seen. The rash cleared after treatment with systemic steroids.

Case 2

A boy aged 5 presented with a 2-week history of a rash. Examination showed an extensive lichenoid eruption with minute vesicles and a few bullae on the chest. The face, scalp, and buccal mucosæ were not affected. There were no nodules. The rash cleared after treatment with antihistaminics and topical steroids.

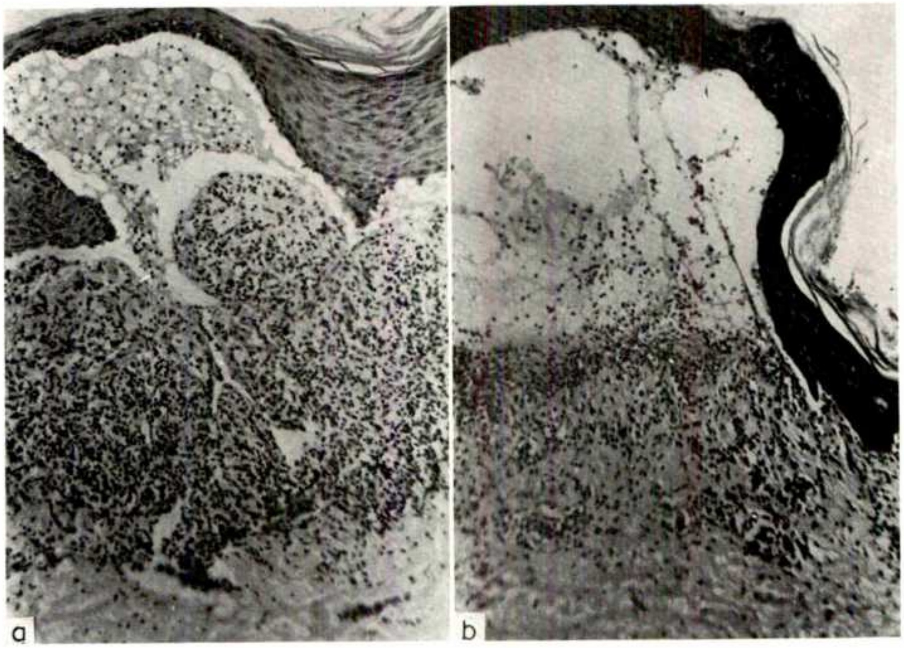

FIGURE 3. Photomicrographs of biopsy of newly formed bulla from skin of breast of patient in Case 1. (a) In the centre of lesion, (b) at the periphery of lesion.

Histopathological examinations

Examination of a bulla from a skin lesion over the breast (Fig. 3a and b) showed a subepidermal bulla, containing serofibrinous exudate and a few inflammatory cells. Examination of a nodule from the leg showed parakeratosis and segmental acanthosis. The rete ridges had splayed sharp points curving inwards. There was oedema of the papillae and upper dermis, with infiltration by histiocytes, lymphocytes, eosinophils and plasma cells, which also infiltrated the basal epidermis. Other lesions that were not bullous or nodular showed the characteristic changes of lichen planus.

INVESTIGATIONS OF DRUGS INGESTED

The first patient, when closely questioned, admitted that she had regularly used a home prepared herbal mixture. This is made by soaking in water for several days the bark, leaves and roots of a certain tree. The extract thus derived was taken orally as medicine, and used in dilution as a bath mixture. The herb used by this patient is widely consumed throughout West Africa, and has the local name of 'Dongoyaro' in the Hausa language, 'Ore' in the Yoruba language, and is *Cinchona tedgeriana*. It has been used by West Africans long before the introduction of conventional medicine, as a home remedy for fever—particularly malarial fever. Both the bark and leaves of cinchona are known to contain quinine as well as other alkaloids (Aviado *et al.*, 1967; Pazzini, 1961).

The second patient had since birth received daily doses of native medicine from his parents who are herbalists. The mixture given to this boy could not be identified.

Patch and provocative tests

A patch test carried out on the boy with the herbal mixture he was used to taking was negative. A provocative test was done with the same drug. 4 days after re-administration there was recurrence of the rash. Both patients (Cases 1 and 2) had further recurrences until finally the native medicine was stopped.

Further evidence in support of the role of native medicine on the incidence of LP in Africa

A special skin clinic was held for members of the university. Medical facilities were free for all members of the university and their families, and a high percentage (as compared to the community outside the campus) sought medical aid when sick. 150 new patients (101 students, 49 staff members and families) attended the skin clinic within 2 months. Only one patient had lichen planus, and he lived outside the campus, and admitted to using native medicine at times, although in this case no direct connection between the lesion and the drug was established. The students living on the campus had no immediate access to herbal drugs. This low incidence of LP is in interesting contrast to the incidence of up to 15% of cases at clinics in other parts of the country.

The role native medicine still plays in Nigeria is revealed by the investigations undertaken by Edington & Maclean (1965). About 58% of 788 families questioned in the Ibadan area admitted to using native medicine. Further, one may say that many of these herbal remedies have been found to be effective in certain diseases and thus they enjoy a widespread clientele. Unfortunately, many of the herbal preparations used in treatment or prophylaxis have not yet been identified, although research in this field is said to be in progress at the University of Ife, Nigeria.

DISCUSSION

The following clinical evidence supports the assumption that the disease in my two cases was lichenoid drug eruption due to the orally and topically administered herbal remedies:

(1) the atypical features of the lesions such as the exanthematous appearance, nodules, obvious hyperpigmentation, and marked scaliness—all are features usually observed in lichenoid drug eruptions;

(2) re-exposure of the patients to the suspected herbal mixture was always followed by an exacerbation or relapse of the rash, and

(3) skin lesions subsided after the suspected drug was completely withdrawn from the patient.

The nodular lesions

Of special interest are the nodes with craters described in Case 1. They were observed often in many of our patients with LP. They contain eosinophils in the cellular infiltrate, with features which accord with those found in lichenoid drug eruptions by Wilson (1946). Hypertrophic lesions especially of the lower limbs are one of the main features of LP among African Negroes, but these umbilicated nodes have not been reported in Africans although umbilicated lichen planus lesions are known (Hellgren, 1967).

It would be premature at this stage to differentiate the umbilicated nodular variant from ordinary hypertrophic lichen planus but it is interesting to speculate that it may be a special feature of drug induced lichen planus in the African.

The absence of buccal mucosa lesions among African Negroes

In 200 cases of LP in Britain, mucous membrane lesions were seen in 73% of cases (Samman, 1962). In all the cases of LP so far observed by us in Nigeria, we have not seen one single case of mucous membrane involvement. Marshall (1969) among others agrees. Moreover, the buccal mucosa is frequently spared in lichenoid drug eruptions (Almeyda *et al.*, 1971). We attribute the absence of buccal mucosal lesions in LP among African Negroes partly to the fact that a great many of the reported cases of LP in Africans could have been due to drugs, traditional and modern, rather than to a genetic factor.

Should one consider the possibility that a few cases of buccal mucosal lesion in African Negroes might be overlooked or misidentified due to the pigmentation? Black (1972) pointed out the role of basal layer and especially the involvement of the melanocyte. Perhaps biopsy of the buccal mucosa of some volunteer African Negroes with and without lichen planus lesions, with comparative studies among the Caucasians, would throw more light on the subject.

The pattern of lichen planus observed in our patients, and also in almost all patients with lichen planus seen in Nigeria, is quite different from that of 'lichen planus tropicus' described by Zawahry (1969). We could not attribute the LP in our patients to the effect of sunlight, and we blame ingested and topically applied native medicine.

ACKNOWLEDGMENT

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Neutron activation analysis of trace elements in skin

VII. COPPER AND ZINC IN VITILIGO, MOLES AND SEBORRHOEIC WARTS

MOHAMED M.MOLOKHIA AND BENJAMIN PORTNOY

Manchester and Salford Hospital for Skin Diseases, Quay Street, Manchester M3 3HL

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SUMMARY

Copper and zinc levels were estimated in serum and skin of nineteen vitiligo patients as well as in pigmented moles, seborrhoeic warts and normal skin. In seborrhoeic warts, high zinc levels were associated with excessive melanization and an abundance of ATPase + ve dendritic cells. Zinc levels were also significantly increased in pigmented moles compared with those in normal surrounding skin.

In vitiligo, there was a significant reduction in serum zinc while copper levels were increased in the hyperpigmented zone surrounding the lesion. The results of copper analysis in vitiligo neither support a deficiency role for copper nor justify its use in the treatment of that disease.

The formation of melanin in skin from the colourless amino acid tyrosine is a specialized enzyme-dependent process. The activity of the enzyme involved, 'tyrosinase', depends on the availability of the trace element copper. Metal binding agents as well as metals competing with copper can inhibit such activity. Chemical analysis of melanosomes has revealed a high zinc content (Seiji *et al.*, 1963) and it was suggested that zinc may play a part in the formation of melanin. However, no zinc-dependent enzymes have been shown to influence the pigmentary system of skin in spite of the fact that the highest levels of zinc in the body are found in pigmented tissue.

There have been very few reports on the status of either copper or zinc in pigmentary disorders and these were in the main limited to changes in blood levels. A significant rise in serum copper levels, similar to that found in pregnancy, has been reported in females taking oral contraceptives (Schenker *et al.*, 1971). Hyperpigmentation of skin is frequently noticed in these circumstances and according to Jelinek (1970), it is the most common cutaneous complication.

High copper levels were also found in the serum of patients with xeroderma pigmentosum (El-Hefnawi, El-Nabawi & El-Hawary, 1962). On the other hand, El-Mofty, El-Hawary & Farag (1961) found that certain cases of vitiligo had subnormal copper levels. However, Kandhari & Sobhandri (1963) could not find any significant alterations from normal in serum copper levels in their cases of vitiligo or hyperpigmentation.

In view of the known antagonism between copper and zinc in biological systems (Underwood, 1971) and the association between these two trace elements and melanin production, the present study was undertaken to assess their possible roles in vitiligo. Samples representing abnormalities of pigmentation were analysed for their copper and zinc contents using the technique of neutron activation analysis.

MATERIALS AND METHODS

Samples of interscapular skin were obtained under local anaesthesia by incision biopsy from sixteen white and two racially dark vitiligo patients who were receiving no treatment at that time. Similar material was obtained from eight racially dark adults as well as from sixteen white volunteers, all with no history of skin disease. Samples taken from the vitiligo patients included adjacent areas of involved and uninvolved skin. Separation of samples into epidermal and dermal components was carried out as described (Molokhia & Portnoy, 1969). Seborrhoeic wart material, collected for a study of dendritic cells (Molokhia & Portnoy, 1971), was also included in this investigation.

Samples of serum were obtained from nineteen vitiligo patients as well as from forty normal subjects who acted as a control group. The methods used for sample preparation and their subsequent irradiation and analysis have been described earlier (Molokhia & Portnoy, 1970b). A combined separation method was used to estimate levels of copper and zinc simultaneously in the analysed material.

RESULTS

Table 1 shows the concentrations of copper and zinc in biopsy material from vitiliginous and normal skin. Although racially dark skin shows slight rises in copper and zinc levels compared with white skin, these are not statistically significant. On the other hand, there is a significant difference in epidermal copper levels between involved and uninvolved vitiliginous skin ($0{\cdot}001 < P < 0{\cdot}01$). A comparison with control skin shows that this difference is caused by a rise in copper levels in the uninvolved epidermis of vitiligo patients. Dermal copper levels are increased in both involved and uninvolved vitiligo skin compared with control, but only those in uninvolved skin reach statistical significance ($0{\cdot}02 < P < 0{\cdot}05$). Among the variations from normal in tissue zinc levels, only the increase shown by involved vitiligo dermis is probably significant ($0{\cdot}01 < P < 0{\cdot}02$).

TABLE 1. Copper and zinc concentrations in vitiliginous and normal skin (μg/g dry weight)

Sample	Copper*		Zinc	
	Epidermis	Dermis	Epidermis	Dermis
	(16)†		(14)	
Control white skin	4·80 ± 1·82	2·08 ± 0 73	56·6 ± 8·8	12·5 ± 3·1
	(8)		(8)	
Racially dark skin	5·04 ± 1·67	2·16 ± 0·85	63·4 ± 14·8	10·3 ± 3·5
Vitiliginous skin	(18)		(15)	
Involved	5·39 ± 1·23	2·61 ± 1·06	48·0 ± 13·4	15·4 ± 3·7
	(18)		(15)	
Uninvolved	8 00 ± 3·24	2·92 ± 1·22	55·1 ± 8·6	13·1 ± 2·8

* Mean concentration ± SD.

† Number of samples.

TABLE 2. Copper and zinc concentrations in the serum of vitiligo patients and normal individuals (μg/g dry weight)

	No. of samples	Maximum	Minimum	Median	Mean	± SD
Copper						
Vitiligo	19	16·40	6·44	11·80	11·25	1·88
Control	39	15·60	5·79	11·20	10·50	2·01
Zinc						
Vitiligo	18	12·44	6·60	9·20	9·10	1·55
Control	40	13·80	7·54	10·10	10·40	1·50

Table 2 shows the results of copper and zinc analysis in serum, calculated on dry weight basis. The mean serum copper level in vitiligo, although slightly elevated, is not significantly different from that in controls ($0·1 < P < 0·2$). On the other hand, vitiligo serum zinc levels show a significant reduction from normal ($0·001 < P < 0·01$). The levels of copper and zinc in control serum are not correlated, whereas those in the vitiligo group show a negative correlation ($r = -0·73$) which is statistically significant ($0·001 < P < 0·01$).

The concentrations of copper and zinc in pigmented moles and surrounding skin are given in Table 3. Copper levels in the epidermis and underlying dermis of these moles were not significantly altered from those of surrounding skin. In contrast, there is a highly significant increase of zinc levels in both the epidermis and dermis of such moles ($P < 0·001$).

Table 4 shows the concentrations of copper and zinc in fifty-six seborrhoeic warts, while Fig. 1 shows the distribution of analytical values in histogram form. Compared with normal skin, these structures contain about 1·5 times as much copper and more than 5 times as much zinc. The heterogenic nature of these lesions is reflected in their wide range of copper and zinc contents. Although up till now there is no satisfactory technique for the quantitative estimation of melanin in tissue, it was clear that a relationship did exist between the degree of melanization of seborrhoeic warts and their zinc content. To assess this point more accurately, seborrhoeic warts showing marked difference in colour in the same individual were analysed for their trace element content (Table 5). Only zinc levels show an increase in dark lesions in every case.

TABLE 3. Copper and zinc concentrations in pigmented moles and their surrounding skin (μg/g dry weight)

	Copper*		Zinc	
	Epidermis	Dermis	Epidermis	Dermis
Mole	(10)† 6·07 ± 1·56	3·82 ± 1·12	(12) 118·9 ± 45·1	43·0 ± 17·6
Surrounding Skin	6·75 ± 2·08	(10) 3·26 ± 1·20	(12) 53·8 ± 10·3	12·0 ± 3·1

* Mean concentration ± SD.

† Number of samples.

TABLE 4. Copper and zinc content of seborrhoeic warts

Element	No. of samples	Concentration in μg/g dry weight				
		Maximum	Minimum	Median	Mean	± SD
Copper	56	11·20	1·92	5·07	5·40	2·05
Zinc	56	396·0	32·2	76·5	96·0	37·2

Some seborrhoeic warts with the lowest copper and/or zinc content were found histologically to consist mainly of horny matter. This finding was verified by the high dry : wet weight ratio in these warts. A comparison between warts with the minimum content of horny matter with those with the maximum is shown in Table 6. The difference between metal content of the two groups is statistically significant ($0·001 < P < 0·01$).

Table 7 shows a comparison between zinc concentrations in seborrhoeic warts with abundant adenosine triphosphatase +ve cells and those without. There is a significant increase in zinc associated with abundant ATPase +ve cells in samples showing similar degree of melanization, both macroscopically and microscopically, to that of the other group.

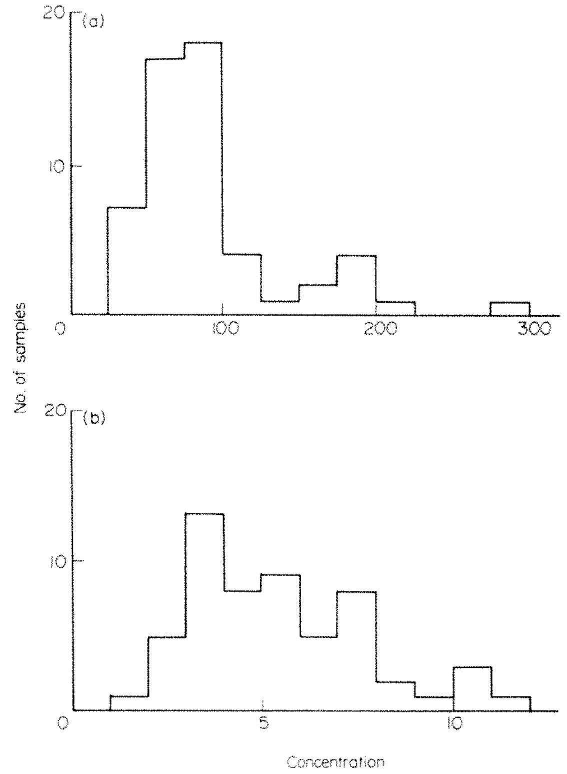

FIGURE 1. Histograms showing the distribution of copper and zinc in seborrhoeic warts (μg/g dry weight). (a) Zinc, (b) copper.

TABLE 5. Variations in copper and zinc levels with pigmentation in patients with multiple seborrhoeic warts (μg/g dry weight)

Sex	Age	Site of lesion	Copper		Zinc	
			Dark	Light	Dark	Light
♀	36	Upper arm	4·70	5·58	76·5	46·2
♂	51	Abdomen	7·20	5·75	98·5	52·0
♂	46	Back	5·08	7·55	161·0	47·6
♀	67	Abdomen	7·45	4·04	134·0	56·8

TABLE 6. Variations in copper and zinc levels with dry-matter content of seborrhoeic warts

Type of lesion	No. of samples	Concentration in μg/g dry weight	
		Copper	Zinc
With less than one-third dry matter per weight	10	6·75 ± 2·04	112·7 ± 40·4
With more than two-thirds dry matter per weight	11	3·91 ± 1·41	60·1 ± 21·5

DISCUSSION

The role of copper as an activator of the dopa-tyrosinase system was discovered by Cunningham (1931) who also found higher copper levels in the skin of black-coated animals compared with white-coated ones. However, Goss & Green (1955) examined black and white hair from ten different species and found no correlation between copper concentrations and the colour of hair. Similar findings in man were reported more recently by Schroeder & Nason (1969) who found no differences in copper levels between black, brown, red or blond hair. These and other findings would suggest that although copper is required for melanogenesis, it does not become incorporated in melanin itself.

Shaffer (1938) suspected that copper may be reduced in vitiliginous skin. Using copper salts in the form of injections or incorporated in ointments, he was, however, unable to induce pigmentation in vitiliginous patches. More promising, although less convincing, results were obtained by El-Mofty

TABLE 7. Zinc analysis in seborrhoeic warts showing similar degrees of pigmentation

Samples	No.	Zinc concentration (μg/g dry weight)		
		Range	Mean	± SD
With no ATP ase + ve cells	10	32·2–98·5	76·4	18·9
With abundant ATP ase + ve cells	9	84·5–396·0	196·6	90·8

$0{\cdot}001 < P < 0{\cdot}01$.

(1957) who found that the addition of copper to local and systemic psoralen therapy and irradiation could increase the percentage of complete cure in vitiligo. In a more recent study of copper metabolism in vitiligo, Huriez (1970) found low blood copper levels in 48% of his patients. However, he could not draw any conclusions as to the therapeutic value of copper in that disease. Although it is conceivable that lack of copper may lead to the absence of tyrosinase activity and, therefore, to lack of melanin, it is difficult to explain why this lack should be confined to certain areas of skin or how copper can correct a structural defect like the absence of melanocytes.

The results of copper analysis in the present investigation neither support a deficiency role for copper nor justify its therapeutic use in vitiligo. Although epidermal copper levels in vitiliginous patches are significantly lower than those found in surrounding involved skin, it is unlikely that this represents a copper deficiency in such lesions. This low copper content in involved epidermis may be due to lack of melanogenesis or absence of melanocytes. On the other hand, the difference in concentrations could have been caused by an actual rise in copper in the hyperpigmented borders of the lesion. The data from the control group suggest that the second explanation is more probable.

Our findings of normal serum copper levels in vitiligo are in agreement with those of Kandhari & Sobhandri (1963). None of these levels was below the range of values found in normal individuals. On the other hand, the results of zinc analysis show that its levels are decreased in vitiligo. The significance of this last finding is not clear.

Heavily pigmented moles and seborrhoeic warts analysed in this investigation were found to contain no more copper than normally pigmented neighbouring tissue. It is zinc that is found in high concentrations in these structures. We have already suggested that the relatively high levels of certain trace elements in the epidermis may be related to its dendritic cell population (Molokhia & Portnoy, 1970a). The finding of high zinc concentrations in seborrhoeic warts with abundant ATPase +ve cells in this study lends further support to the above hypothesis. A study of trace element levels and dendritic cell counts in epidermal sheets from lesions like pigmented moles may reveal the true nature of such a relationship.

ACKNOWLEDGMENTS

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Glycosaminoglycans in human skin

J.M.J.VAN LIS, T.KRUISWIJK, W.H.MAGER AND G.L.KALSBEEK

Department of Dermatology, Free University Hospital, De Boelelaan 1117, Amsterdam, The Netherlands

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SUMMARY

Small skin biopsies of thirty-nine human subjects were assayed for the qualitative and quantitative analysis of glycosaminoglycans.

The following results were obtained:

(1) Besides the presence of hyaluronic acid and dermatan sulphate, evidence has been presented for the presence of heparan sulphate.

(2) The content of glycosaminoglycans was higher in biopsies from healthy individuals than the values reported for post-mortem human skin.

(3) Variation of the concentration of either hyaluronic acid or dermatan sulphate plus heparan sulphate in relation to the region of the body could not be demonstrated.

(4) With advancing age a decrease in dermatan sulphate plus heparan sulphate could be demonstrated. However, hyaluronic acid content could not be demonstrated to correlate with age.

The major glycosaminoglycans (acid mucopolysaccharides) of adult human skin have been shown to be hyaluronic acid and dermatan sulphate. Moreover foetal skin contains chondroitin 4-sulphate and/or chondroitin 6-sulphate in substantial quantities (Loewi, 1961; Breen *et al.*, 1970a).

The purpose of the present investigation was to perform a quantitative and qualitative analysis of the glycosaminoglycans in biopsies of human skin.

Furthermore, we wished to establish the variation in the content of the glycosaminoglycans in relation to age and skin site. Human skin was analysed with a micromethod as described by Mier & Wood (1969a). Indentification of the glycosaminoglycans was done with electrophoresis on cellulose acetate strips.

EXPERIMENTAL

Materials and methods

Most specimens of the skin were obtained from the surgical department of our hospital. The specimens from the back were biopsies taken from normal skin of patients attending the dermatological department of the hospital.

Punch biopsies with a diameter of 3 or 4 mm were performed after freezing the skin with ethyl chloride. After a short wash with 0·9% NaCl the specimens were dried at room temperature, the fresh weight was determined, and the specimens were stored at −80°C until use.

The glycosaminoglycans were isolated by the method of Mier & Wood (1969a). This technique is based upon the precipitation of the glycosaminoglycans as a complex with cetylpyridinium chloride in the presence of the coprecipitant celite (final concentration about 0·1%), followed by extraction successively with 0·4 M, 1·2 M and 2·1 M NaCl. The fractions, to which a known quantity of D-glucuronic acid (10 μg) was added, were assayed for uronic acid, using the carbazole method of Bitter & Muir (1962).

The dry defatted weight of the biopsies ranged from 4 to 20 mg. Electrophoretic separation was done on cellulose acetate strips (25·5 × 145 mm; Sartorius-membranfilter GmBH) to identify the glycosaminoglycans in the three extracts. 1 ml of each fraction was evaporated at 50°C to dryness by blowing air over the surface. The remaining solid was dissolved in 10 μl 2·1 M NaCl. About 1 μl of this solution was applied to the strip.

The runs were performed in 25 mmol copper (II) acetate–acetic acid pH 3·6 for 70 min (Stefanovich & Gore, 1967), 0·2 M $ZnSO_4$ pH 5·2 for 30 min (Breen *et al.*, 1970b) or in Michaelis buffer pH 8·6 for 40 min (Wessler, 1968). All runs were done at 1·5 mA per strip.

The strips were stained for 8 min in 1% Alcian blue in ethanol–0·2 M sodium acetate (1 : 1, v/v)pH 3·5. Excess of stain was removed by rinsing with 5% acetic acid in 10% ethanol. The action of hyaluronidase on the glycosaminoglycans was investigated as follows:

1 ml 0·1 M sodium acetate buffer pH 6·1, containing 0·83 mg hyaluronidase (i.e. 282 NF units) was added to 1 ml sample. The mixture was incubated for 20 h at 37°C. After this procedure electrophoresis was performed as described. Standard glycosaminoglycan solutions used for electrophoresis were made of hyaluronic acid from human umbilical cord, chondroitin 4-sulphate from whale cartilage, dermatan sulphate from pig skin, chondroitin 6-sulphate from shark cartilage and heparan sulphate from bovine aorta (heparan sulphate was obtained as a generous gift from Dr J. A. Cifonelli, Department of Pediatrics, University of Chicago. All other materials were obtained commercially).

RESULTS

Identification of the glycosaminoglycans

Fourteen samples of the three NaCl fractions were examined by electrophoresis.

The 0·4 M extract contained hyaluronic acid. The 1·2 M extract showed two bands corresponding to dermatan sulphate and heparan sulphate (see Fig. 1). Two samples showed an electrophoretic mobility, slightly less than chondroitin 4-sulphate and chondroitin 6-sulphate. This faint staining was found only when zinc sulphate was used, but not with copper II acetate. The 2·1 M extract contained 6–19% of the total glycosaminoglycans which appeared to be merely dermatan sulphate. This component co-ran with the dermatan sulphate of the 1·2 M extract.

To confirm the identity of the heparan sulphate found, the action of hyaluronidase on the several glycosaminoglycans was investigated. In control experiments hyaluronic acid, chondroitin 4-sulphate and chondroitin 6-sulphate appeared to be disintegrated completely. However, dermatan sulphate and heparan sulphate were both relatively resistant.

The observed specificity of hyaluronidase is in accordance with the literature (Linker *et al.*, 1958; Meyer, 1969). The 0·4, 1·2 and 2·1 M fractions were pooled and incubated with the enzyme. Electrophoresis showed two bands corresponding to dermatan sulphate and heparan sulphate. So our tentative conclusion of the presence of heparan sulphate in human skin was confirmed.

Quantitative results

When the 1·2 M extract was refractionated a small fraction of the dermatan sulphate appeared in the 2·1 M extract. From this it may be concluded that the fractionation procedure used is not sufficient

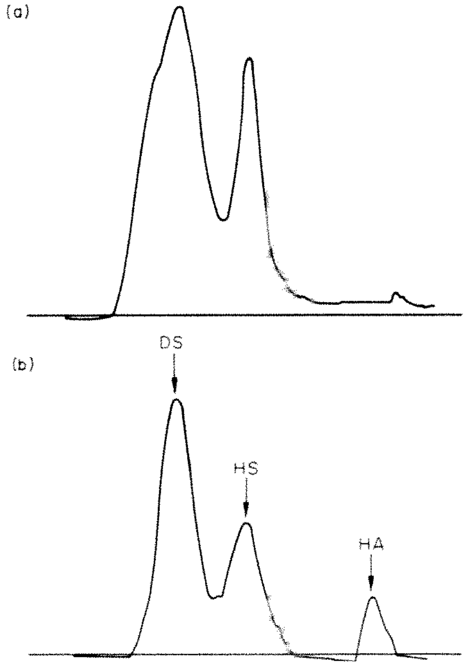

FIGURE 1. Electrophoresis of glycosaminoglycans in Michaelis buffer. The strips were scanned in a densitometer. Mobility is from right to left. (a) The components of the 1·2 M NaCl fraction. (b) A mixture of the three glycosaminoglycan standards. HA, hyaluronic acid; HS, heparan sulphate; DS, dermatan sulphate.

to obtain dermatan sulphate exclusively in one single fraction. We decided to combine the 1·2 M fraction and the 2·1 fraction. Thus our quantitative results are concerned with the values of hyaluronic acid (0·4 M fraction) and dermatan sulphate plus heparan sulphate (1·2 + 2·1 M fraction).

Reliability of the procedure

The glycosaminoglycans were determined in eight biopsies taken from a single piece of skin. The dry weight of each biopsy was approximately 9 mg. The mean content of hyaluronic acid and dermatan sulphate plus heparan sulphate, together with the standard deviations, are shown in Table 1. The values are expressed as micrograms uronic acid per gram dry weight, as well as per gram fresh weight. From Table 1 it can be seen that the coefficient of variation (i.e. the standard deviation divided by the mean) is approximately the same (0·15) for data expressed in terms of both fresh and dry weight.

TABLE 1. Reliability of the assay procedure. Mean values and standard deviations (SD) in μg uronic acid/g dry and fresh weight

	Dry weight		Fresh weight	
	Mean	SD	Mean	SD
Hyaluronic acid	736	111	245	38
Dermatan sulphate + heparan sulphate	633	83	211	32

TABLE 2. Glycosaminoglycans in different areas of skin. Mean values and standard deviations (SD) in μg uronic acid /g dry weight

	No. of specimens	Hyaluronic acid		Dermatan sulphate + heparan sulphate	
		Mean	SD	Mean	SD
Back	6	820	125	549	76
Breast	8	877	73	767	171
Abdomen	10	772	204	679	241
Leg	7	912	147	724	118
Prepuce	8	1109	187	1288	371

Variation with site of skin

Thirty-nine specimens from five different areas of the body were analysed. The mean concentrations of hyaluronic acid and dermatan sulphate + heparan sulphate, together with the standard deviations are presented in Tables 2 and 3. All values are expressed as micrograms uronic acid per gram dry weight as well as per gram fresh weight.

The distributions of the values measured do not deviate significantly from the Gaussian distribution (see Shapiro & Wilk, 1965). Thus analysis of variance and covariance (to take into account variations in relation to age), followed by multiple comparisons (according to Kramer, 1956), were used to test whether the mean concentrations for the five areas differ from each other. No differences were found; only the mean concentrations of both glycosaminoglycans per gram dry weight for the prepuce differed from the mean concentrations for the other four areas ($P < 0{\cdot}01$).

The tests were performed at the 5% level of significance.

Correlation with age

The specimens were from individuals (twelve men and twenty-seven women), ranging in age from 3 to 77 years. The correlation coefficients of the content of the glycosaminoglycans with advancing age were calculated for the five areas, from the values shown in Fig. 2. Those for the back are positive, but only significantly so when the concentrations are expressed in terms of dry weight. Those for the

TABLE 3. Glycosaminoglycans in different areas of skin. Mean values and standard deviations (SD) in μg uronic acid /g fresh weight

	No. of specimens	Hyaluronic acid		Dermatan sulphate + heparan sulphate	
		Mean	SD	Mean	SD
Back	6	226	21	152	16
Breast	8	252	34	220	50
Abdomen	10	238	79	207	77
Leg	7	261	55	207	43
Prepuce	8	234	58	260	74

other four areas cannot be demonstrated to differ from each other. Therefore, the combined correlation coefficients for leg, breast, abdomen and prepuce were calculated. The results are presented in Table 4.

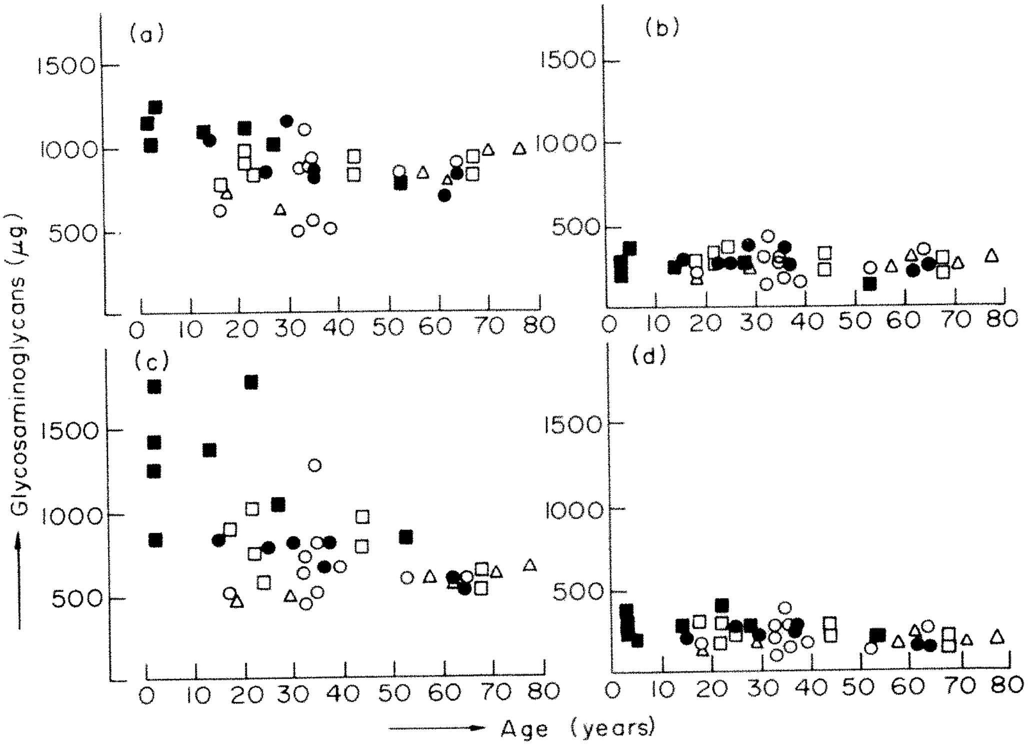

FIGURE 2. Correlation of glycosaminoglycans with age. (a) Hyaluronic acid per dry weight versus age. (b) Hyaluronic acid per wet weight versus age. (c) Dermatan sulphate + heparan sulphate per dry weight versus age. (d) Dermatan sulphate + heparan sulphate per wet weight versus age. □, Breast; ■, prepuce; ○, abdomen; ●, leg; △, back.

TABLE 4. Correlation coefficients with age, combined for breast, abdomen, leg and prepuce

	Dry weight	Fresh weight
Hyaluronic acid	−0·33	−0·38
Dermatan sulphate + heparan sulphate	−0·52	−0·52

From Table 4 we concluded that there is a negative correlation with age for dermatan sulphate plus heparan sulphate ($P < 0{\cdot}05$). We could not demonstrate a correlation between hyaluronic acid and age. Again the tests were performed at the 5% level of significance.

DISCUSSION

Glycosaminoglycans were analysed both qualitatively and quantitatively in small biopsies of human skin. We demonstrated the presence of hyaluronic acid, dermatan sulphate and heparan sulphate. However, in our fractionation procedure no separation could be effected between heparan sulphate and dermatan sulphate. Loewi (1961) and Breen *et al.* (1970b), using post-mortem material, were unable to detect heparan sulphate in human skin. On the contrary, they found chondroitin 4-sulphate and/or chondroitin 6-sulphate. We could not positively identify them. Fabianek, Herp & Pigman (1965) as well as Mier & Wood (1969b) detected in the 2·1 M NaCl fraction a small amount of glycosaminoglycans. They reported this to be heparin without any proof. Analysing the 2·1 M fraction we demonstrated only the presence of dermatan sulphate. This does not contradict the findings of Schiller, Slover & Dorfman (1961) and of Smith, Davidson & Taylor (1964). These authors reported chondroitin sulphate in the 2·1 M fraction.

We observed that the content of glycosaminoglycans was higher in biopsies from healthy individuals than the values reported for post-mortem human skin (Smith *et al.*, 1964; Fabianek *et al.*, 1965; Mier & Wood, 1969b). Our results are in agreement with those of Hollman *et al.* (1971) who analysed biopsies from the upper arm and the trunk. However, these authors neglected the 2·1 M NaCl fraction, resulting in a lower total dermatan sulphate level.

We could not find differences in the content of glycosaminoglycans between the various areas of the body, with the exception of the prepuce. Mier & Wood (1969b) concluded the same with respect to abdomen, leg, arm, sole and scalp. Clausen (1962) reported a marked decrease of the total glycosaminoglycan content in abdominal skin with advancing age. Meyer (1969) stated that there was a fall in the level of hyaluronic acid in this site with advancing age. Combining the values for breast, abdomen, leg and prepuce, we found a negative correlation between dermatan sulphate plus heparan sulphate and age. However, hyaluronic acid could not be demonstrated to correlate with age. The positive correlation between the values of back skin and advancing age is difficult to interpret.

The method used, enabled us to analyse the glycosaminoglycans in small skin biopsies qualitatively and quantitatively.

The present results provide a valuable tool for the detection and evaluation of changes in the content of glycosaminoglycans of the skin in various dermatoses.

In preliminary studies we found a marked increase of hyaluronic acid in skin lesions of localized (pretibial) myxoedema.

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The epidermal response to subepidermal inflammation

AN EXPERIMENTAL STUDY

F. SCHELLANDER AND R. MARKS

Department of Pathology, The Institute of Dermatology,
Lisle Street, Leicester Square, London, W.C.2

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SUMMARY

A marked degree of hyperproliferation and hyperplasia of the epidermis secondary to experimentally induced inflammation in the corium was induced in the flank skin of guinea pigs. The substances used to evoke a granulomatous inflammation were beryllium oxide and carrageenan. The epidermal response to this subepidermal process was studied autoradiographically and it was found that the epidermal reaction was similar to that found after direct epidermal trauma such as stripping of the horny layer. It is assumed that dermal factors are responsible for initiating this epidermal reaction and their possible nature is briefly discussed.

Hyperplasia of the epidermis is a morphological expression of epidermal reactivity. The form it takes may vary and there is also some indication that a number of different factors may be operative in producing it.

A number of investigations have studied experimentally produced epidermal hyperplasia, most of them using direct epidermal stimulation such as mechanical disruption, stripping with adhesive tape, the painting on of irritant substances or simple rubbing (Hell & Cruikshank, 1963; Schellander, 1969; Steigleder & Gans, 1964; Christophers, 1968). Consequently, a great deal is known about epidermal thickening due to direct stimulation.

Epidermal hyperplasia, however, is found not only as a reaction to direct epidermal trauma, but also where the primary pathological process is mainly dermal and the epidermis seems to be involved only secondarily. This situation is illustrated by the various morphological types of epidermal hyperplasia seen overlying histiocytoma, granular cell myoblastoma, warty tuberculosis or blastomycosis. Very little seems to be known concerning the way this hyperplasia develops or what causes it despite the ever expanding knowledge in the field of dermo-epidermal interactions.

It was the purpose of this study to produce epidermal hyperplasia secondary to experimentally induced inflammation in the dermis of guinea-pigs and to study the morphodynamic events in the epidermis above it.

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* Based on a paper read at the Annual Meeting of the British Association of Dermatology, July 1972.

MATERIAL AND METHODS

Albino guinea-pigs were used. Sterile suspensions of 10% beryllium oxide in isotonic saline, and 1% saline solution of carregeenan, a polysaccharide extract of seaweed (Sigma Chemicals Ltd), were injected into the skin of the flanks to produce a granulomatous inflammation. Four to five injections of each substance were performed on either side of each animal. The amounts injected varied from 0·1 to 0·5 ml according to the depth of the injection. 1, 2, 4, 8 and 15 days later tritiated thymidine (1 μCi/g body weight, sp. act. 26 Ci/mmole, Radiochemicals, Amersham) was injected intraperitoneally and the animals were sacrificed 60 min thereafter. The areas bearing the injection sites were excised *in toto* and fixed in 10% neutral formalin and then divided into separate biopsies which were embedded in paraffin. From this material 5 μm thick sections were prepared and processed autoradiographically by dipping in Kodak NTB2 nuclear emulsion. The slides were developed and fixed after 3–5 weeks exposure at 4°C. All sections were stained through the emulsion with haematoxylin and eosin. In addition, carregeenan was identified in the section by its ability to stain metachromatically with toluidine blue. All autoradiographs were evaluated as follows:

(1) The rate of epidermal proliferation was expressed as the 'labelling index', i.e. the ratio between the number of keratinocytes labelled in the period of DNA synthesis with thymidine and the total number of labelled and unlabelled basal cells. Between 1000 and 2000 basal cells were counted in each specimen. Labelled Langerhans cells were, whenever possible, excluded from the counts by a method described elsewhere (Schellander & Marks, 1973).

(2) Epidermal thickness was measured from drawings of sections projected onto a screen at a standard magnification. On average, 100 measurements of each specimen were made.

(3) The degree of papillomatosis was estimated by calculating the ratio between the length of the granular cell layer and the basement membrane zone from specimens projected in the same way as above. Normal guinea-pig flank skin was used for control observations and care was taken to avoid the vicinity of the epidermal defect caused by injection.

RESULTS

Epidermal reaction

Normal guinea-pig flank skin always has a thin epidermis consisting of three to four cell layers and virtually no rete ridge pattern. Labelled keratinocytes are normally present in the basal cell layer only and in these studies the labelling index was found to be in the region of 4% (Fig. 1).

The reactions in the epidermis following the injection of carregeenan and beryllium oxide were of the same degree and kind and will therefore be discussed together. 24 h after the injection of the two substances an acute inflammatory reaction was present around the deposits of the injected materials. At this stage the epidermal morphology appeared unchanged, however, the labelling index had risen to more than 30% with the majority of labelled cells in a basal position. 2 days after the injection there was pronounced epidermal oedema with keratinocytes arranged in a disorderly fashion. The keratinocyte labelling index had risen to 20% and epidermal thickness was 2·5 times the normal values (Fig. 2). Labelled keratinocyte nuclei were present in the lower two cell layers of the epidermis. On close inspection, however, it became apparent that many of them were in fact, nuclei of elongated basal cells which were still tethered to the basement membrane. At this stage the increase in epidermal thickness was partly due to the oedema and partly due to a real increase in the number of cells. This early reaction was strikingly similar to that found after stripping of the horny layer with adhesive tape, with the exception that parakeratosis never developed.

After 4 days the epidermal thickness had increased to more than 3 times the normal value as a

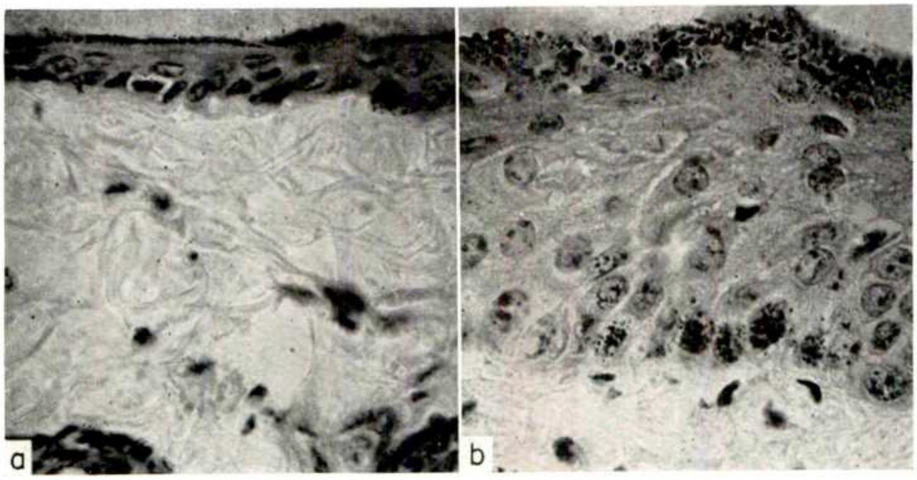

FIGURE 1. (a) Normal guinea pig flank skin. Epidermis three to four cell layer thick. Labelled cell in the basal layer. (b) Maximal acanthosis as found 4 days after trauma. Malphigian cells appear swollen and oedematous. Labelled cells mostly in the basal cell layer (H & E, × 150).

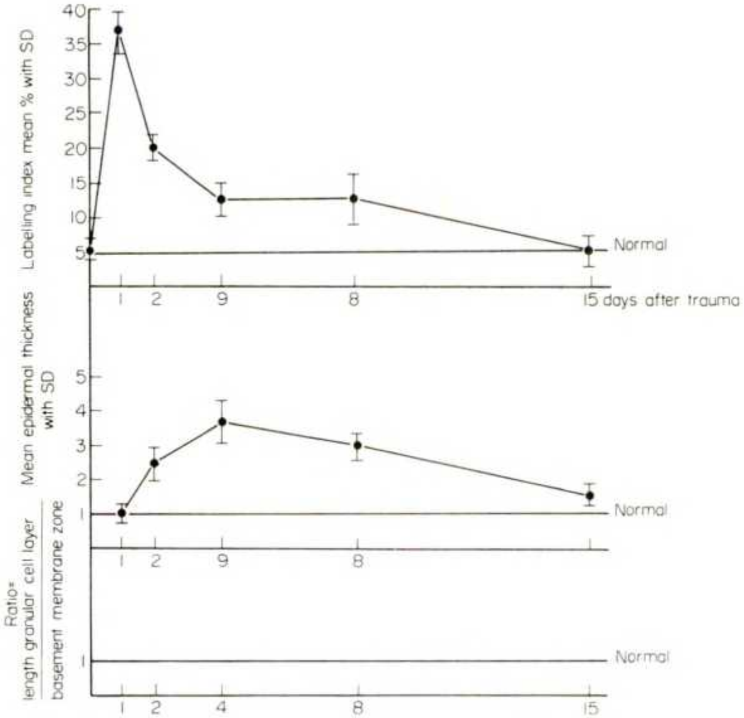

FIGURE 2. Diagram to show relationship between labelling indices, epidermal measurements and time.

result of the considerable increase in the number of Malpighian cells during the previous days. The labelling index at this stage was still considerably raised (12%) but less than that at 2 days earlier. The oedema was less and labelled keratinocytes were again limited to the basal layer (Fig. 1).

At day 8 the labelling index was still 12% but epidermal thickness was less than at day 4 (Fig. 2).

At day 15 the situation was near normal with only slightly raised indices and only a 1·5 fold increase in epidermal thickness. At no stage did any degree of hypertrophy of the papillary bodies develop (Fig. 2), and the ratio between the length of the granular cell layer and the basement membrane zone always remained approximately one.

From day 4 a considerable number of labelled 'high-level' cells were present in the acanthotic epithelium. These were, on the basis of various criteria, assumed to be Langerhans cells and their number was deducted from the total epidermal cell counts (see above in Material and Methods).

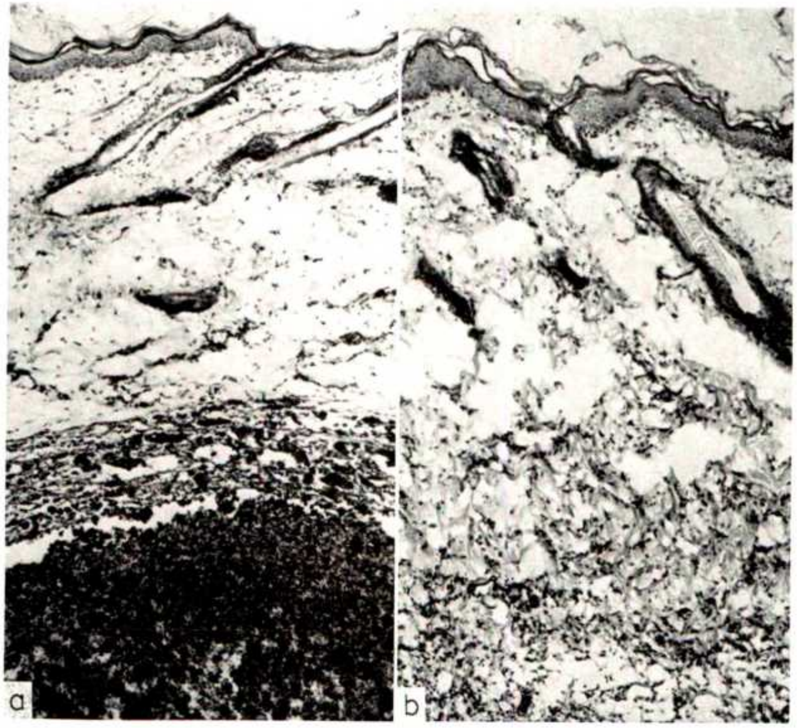

FIGURE 3. (a) Encapsulated beryllium granuloma—no response in the overlying epidermis. (b) Diffuse granuloma response after carregeenan injection—hyperplasia of the epidermis. 8 days after the injection in both cases (H & E, × 175).

Significance of depth of infiltrate

It was evident that where the inflammatory infiltrate did not extend above the level of the hair papillae no epidermal reaction took place (inflammation in the subcutaneous fat never led to any epidermal reaction). This applied to the acute and more chronic stages of the induced inflammation. Only diffuse infiltrates were capable of evoking a response. When new connective tissue encapsulated the area of inflammation no epidermal reaction was seen (Fig. 3).

The hyperplasia was most pronounced in the epidermis just above the inflammatory focus, but tapered off at the edges, so that slight hyperplasia would be present over apparently normal dermis.

DISCUSSION

We cannot trace any previous work in which an attempt had been made to induce hyperplasia of the epidermis through inflammation in the dermis. The experimental model described here shows that hyperplasia of the epidermis can be induced in guinea-pigs simply by injection of foreign material into the corium.

The epidermis reacts vigorously to the early granulomatous inflammation in a manner which closely resembles the response to primary epidermal damage such as that caused by stripping of the horny layer with adhesive tape (Schellander, 1969). However, it is unlikely that direct damage to the epidermis from the injected materials was the cause for the considerable oedema and hyperproliferation observed, as these two substances were deposited in the mid-dermis and were not in contact with the epidermal undersurface.

In the light of the present knowledge concerning dermo-epidermal interactions it is difficult to explain the cause of the epidermal reaction described in this experiment. This is especially so as the cellular response to the injected materials is very complex due to the presence of monocytes and fibroblasts as well as polymorphonuclear cells in the early stages of the inflammation. There are however several theoretical possibilities including the release of a dermal mitotic stimulator (Karasek & Charlton, 1971), a change in vascular permeability with disturbance of the epidermal capillary unit (Ryan, 1969), or even a slight degree of epidermal hypoxia as a result of the inflammatory reaction may be responsible (Steigleder & Gans, 1964). However, this study was primarily concerned with kinetic aspects of the epidermal reaction and further studies will be required before more insight is gained into the mechanisms responsible for the reaction pattern described here.

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Short life of stimulant released from ground skin slices

ELIZABETH HELL AND J.COOPER

MRC Unit on the Experimental Pathology of Skin,
The Medical School, The University, Birmingham 15

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SUMMARY

Ground guinea-pig skin slices release a potent stimulant to epidermal cells which causes them to enter DNA synthesis (Hell, 1970). Further experiments have shown that this effect on the epidermis is immediate. If the stimulant-containing medium is replaced 15 min after setting up the experiment by fresh medium containing no stimulant, the epidermal cells have already received the impetus to enter the DNA synthetic period, and the original medium is without its characteristic effect on a further set of skin slices.

Professor Lajtha said in 1967 'The outstanding problem is the mechanisms which govern the steady states. From models, we can surmise possible regulating processes, but there is a profound lack of experimental evidence on their nature and of the substances involved. This may be due, in part, to the short life of such regulating substances—a short life-span of these is a prerequisite of a sensitive regulation—and partly to the relative crudity of biochemical separating methods available'. A potent stimulant to epidermal cells causing them to enter DNA synthesis had previously been demonstrated (Hell, 1970). Because of its instability, identification is proving very difficult. However, this very property is what would be expected of such a material if it is part of a sensitive regulating system. The experiments reported in this paper were designed to see if this property was real or apparent: would it be able to stimulate a second set of skin slices at a time when the activity would, on past experience, be expected to be considerably diminished, and would this short length of time when it could be assumed to be present, be long enough for the triggering effect to occur. Of course, it could simply be 'used up' in the first part of the experiment, but then one could reasonably expect a concentration effect to become apparent in the second part. In general, the mechanisms of this stimulant effect appears to be an 'all or nothing' response: if 1/100 concentration gives an increase over the control of 100%, 1/25 does not raise this increase to 400%. There is only a slight increase with increasing concentration. Perhaps there is/are inhibitor(s) present. Or there may be an optimum concentration.

METHODS

Skin slices were cut free-hand from the dorsa of the ears of freshly killed albino guinea-pigs. The specimens consisted of epidermis with just enough dermis to make sure of having all the epidermal basal layer. Those which were to be used for preparing skin extract were ground immediately in a

Pyrex Potter grinder pre-cooled in ice–salt mixture, washed out with cold tissue culture medium, approximately 1 ml/10 mg, centrifuged for 6 min at 3000 rev/min and 0°C, and dilutions made as quickly as possible from this supernatant with the rest of the tissue culture medium. This was Hanks' (1948) balanced salts solution/horse serum 3 : 2 containing ^{3}H thymidine 5 μCi/ml, 36 μCi/μmol. The concentrations of extract were 1/100, 1/50, 1/25, and controls were always included, all observations being made on duplicate skin slices, each in its own culture bottle, floating dermis side down on 1 ml medium. After 15 min incubation at 37°C, the skin slices were transferred to fresh pre-warmed tissue culture medium in other bottles, not containing any of the skin extract, and incubation continued for the balance of 4 h. A fresh set of skin slices were, at the time of transfer, put on the original medium in nine experiments and also incubated for 4 h. After incubation, the medium was replaced with buffered formal saline and the skin slices processed as described previously (Hell & Cruickshank, 1963). The autoradiographs were exposed for 3 days, developed and stained, and counts of at least 2000 epidermal cells per skin slice made; control and 'stimulated' were compared after averaging the results of each pair.

RESULTS

In twelve experiments there was significant stimulation of the proportion of cells in DNA synthesis in the skin slices which had the fresh wound hormone. Results are contained in Table 1. In the nine experiments of these where a second set of skin slices (from another animal) were put on the original medium after fifteen minutes, there was no stimulus (Table 2). Both sets of results are summarized in Fig. 1, which gives the averages of the observed effects at each concentration. The results are slightly lower in the 'second-hand' medium which had contained skin extract than in the 'second-hand' control medium, but not significantly so.

TABLE 1. Percentage of epidermal cells in DNA synthesis after 15 min in medium containing stimulant and 3¾ h in medium not containing stimulant

	Averages of duplicate			
Exp. no.	Control	1/100	1/50	1/25
1	4·7	6·1	4·4	5·4
2	1·5	3·3	2·2	4·8
3	6·5	7·3	13·0	7·9
4	5·9	6·1	6·3	8·6
5	4·4	6·3	6·0	5·2
6	8·1	11·4	19·9	13·5
7	8·4	6·1	9·9	9·0
8	7·1	9·6	6·9	5·1
9	4·7	5·9	12·8	12·9
10	5·4	8·4	6·3	6·1
11	4·9	7·2	7·1	8·8
12	4·8	8·2	10·2	6·7
Mean	5·3 ± 1·9	7·2 ± 2·1	8·8 ± 4·0	7·8 ± 3·1

TABLE 2. Percentage of epidermal cells in DNA synthesis after 4 hr in 'second-hand' stimulant media

	Averages of duplicates			
Exp. no.	Control	1/100	1/50	1/25
4	5·4	4·0	6·9	4·0
5	8·1	7·7	7·4	7·9
6	11·4	9·1	8·4	9·5
7	9·3	9·5	8·3	8·5
8	6·0	5·9	4·5	5·7
9	4·3	2·6	2·6	2·6
10	5·1	6·7	6·4	4·0
11	7·3	4·9	6·3	5·4
12	7·2	7·8	5·7	9·8
Mean	7·1 ± 2·2	6·5 ± 2·3	6·3 ± 1·9	6·4 ± 2·6

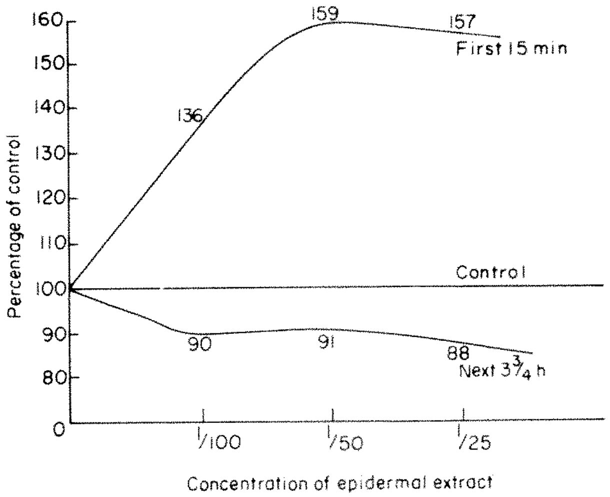

FIGURE 1. The effect of different concentrations of wound hormone on the percentage of epidermal cells in DNA synthesis.

DISCUSSION

The unstable stimulant does seem to be capable of pushing extra epidermal cells into DNA synthesis in 15 min of contact. After that time, it is not available for fresh pieces of skin to be stimulated by it. This would account for the observed synchrony of the extra population of cells brought into the cell cycle as a result of injury. Lack of an inhibitor would be expected to give no such synchrony, unless it was similarly extremely unstable, which epidermal chalones do not appear to be. Of course, it is always possible to suppose that this stimulant we have found, acts as an unstable inhibitor of an inhibitor (chalone) but this does seem unnecessarily complicated. The stimulant idea is a simpler concept.

The second set of skin slices were not stimulated; in fact, there may even have been a little inhibition, which was not eonugh to be measured in these experiments. Or it may be that initiation of DNA synthesis has altered the medium in some way, making it slightly less capable of supporting cells in DNA synthesis. However, it is altogether more likely that, the stimulant having been removed, there remains a mild inhibitor. Compared with chalone experiments (Bullough & Laurence, 1964; Iverson, Aandahl & Elgjo, 1965), these experiments show the effect of a remarkably small amount of extract.

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Pharmacology and Treatment

Plasma corticosteroid levels in out-patients treated with topical steroids

LYN WILSON, D.I.WILLIAMS AND STEPHANIE D.MARSH

Clinical Research Unit, Glaxo Laboratories Ltd, Greenford, Middlesex
and King's College Hospital, Denmark Hill, London

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SUMMARY

Plasma corticosteroid levels in 295 out-patients using halogenated topical steroids were compared with levels in 158 controls. The mean level of the treated patients was 12·5 μg/100 ml compared with 14 μg/100 ml in the controls; 96% of the patients who used topical steroids and 99% of the controls had levels of 6 μg/100 ml or more. Five of the six patients whose plasma corticosteroid levels were less than 5 μg/100 ml on first sampling had normal values on retesting after an interval of 2 weeks or more.

The effects of drugs are often studied in ways quite different from those used in ordinary clinical practice. It is important to have the sort of information about a drug which can be gathered by observing small numbers of patients in hospital where measured doses can be given under supervision but it may be difficult to know how to interpret the results of such a study when it comes to treating patients under everyday conditions.

There is ample evidence that those topical steroids which are highly effective clinically are capable of being absorbed through the skin to an extent that is detectable by changes in endogenous corticosteroid production (Scoggins, 1962; Kirketerp, 1964; James, Munro & Feiwel, 1967; Munro, Feiwel & James, 1967). But each of these studies was performed under conditions which are rarely used in everyday practice. Munro, for instance, studied in-patients with 60% or more of their body surface affected by skin disease, and applied 30 g of steroid ointment a day for 14 days; whole body occlusion was used for 20 h out of each 24. Yet the vast majority of people who are prescribed topical steroids are treated as out-patients and use considerably less than 30 g of steroid preparation a day. Occlusive dressings, if used at all, are likely to be applied to circumscribed areas and only overnight.

There is remarkably little reliable evidence of adverse systemic effects resulting from steroid absorption through the skin. Sporadic reports have been published concerning growth retardation (Benson & Pharaoh, 1960; Fanconi, 1962) and describing oedema following the intensive use of topical hydrocortisone in children (Feinblatt *et al.*, 1966); Keipert & Kelly (1971) described an 11-week-old baby who developed a Cushingoid appearance after the application of large amounts of betamethasone 17-valerate cream. We can find no reference to an adverse systemic effect occurring in an adult. Nevertheless, most doctors feel concern at the possibility of placing any patient at risk. We have, therefore, tried to find out the extent to which topical steroids affect patients' circulating corticosteroid level under the ordinary conditions of treatment met in a hospital out-patient department.

METHOD

Patients

The treated group consisted of out-patients over the age of 10 years who were being treated with halogenated topical steroids. The control group consisted of out-patients over the age of 10 years for whose skin condition topical steroids of any sort had not been prescribed and who had no recent history of having used topical steroids. The following were excluded from both groups: pregnant women; patients receiving systemic steroids (including oral contraceptive tablets); those who had had systemic steroids within the previous 3 months; and those others whose disease or treatment was known to affect hypothalamic-pituitary-adrenal status.

Interview

All the patients were interviewed by one of us (SDM). Diagnoses and relevant details were recorded and patients were carefully questioned about their drug history. Patients in the treated group were asked about their topical steroid regimen and details of the type of steroid and amount used per week, the duration of use and whether or not occlusive dressings were employed were recorded. Whenever possible the answers given by the patients at interview were cross-checked with the patients' hospital records.

Plasma corticosteroid estimations

At the time of interview 10 ml of venous blood was collected into an heparinized tube. The blood was withdrawn before the patient underwent any procedure, such as the use of liquid nitrogen for warts, that might cause stress. The time at which the sample was taken was recorded. Plasma corticosteroid levels were estimated by the method of Spencer-Peet, Daly & Smith (1965); duplicate estimations were carried out on each of the samples which were coded so that it was not known whether they were from treated or control patients.

RESULTS

Composition of the groups

The treated group comprised 295 patients and 158 patients formed the control group.

Table 1 illustrates the comparability of the two groups in respect of age and sex. The control group includes a higher proportion of the younger age segments but this is not thought to be of significance as there is no evidence that plasma corticosteroid levels are age-dependent after the first few weeks of life.

Diagnosis

In the steroid-treated group 41% of the patients had eczema, 33% psoriasis and the rest various other steroid-responsive skin diseases. The largest disease categories in the control group were acne vulgaris and rosacea, each accounting for 25%, and 15% were being treated for warts; other conditions included fungal infection of the skin, leg ulcer and basal cell carcinoma.

Topical steroid treatment

The amount of topical steroid preparation used by patients in the treated group is shown in Table 2.

Quantities are expressed as grams per week of the manufacturer's formulated steroid—no patient received diluted steroid preparations. Ninety percent of the patients were being treated with betamethasone 17-valerate. The remainder were using preparations of one of the following halogenated steroids: beclomethasone diproprionate, dexamethasone, fluocinolone acetonide, fluocinonide, fluocortolone and fluocortolone caproate, formocortal or triamcinolone acetonide. Table 2 also shows the numbers and proportion of patients who were using occlusive dressings. Polythene occlusion was used only overnight and in the main either on hands or feet. Impregnated dressings, most often Quinaband, were used only on the limbs and remained undisturbed for 7 days.

TABLE 1. Comparison of sex and age distributions of treated and control groups

	Control patients (%)			Treated patients (%)		
Age	Men	Women	Total	Men	Women	Total
10–19	8·2	6·3	14·6	4·4	4·8	9·2
20–29	13·3	12·0	25·3	7·5	4·8	12·2
30–39	6·3	5·1	11·4	6·4	3·4	9·8
40–49	8·9	5·7	14·6	8·5	7·8	16·3
50–59	7·6	8·2	15·8	8·5	10·2	18·6
60–69	6·3	4·4	10·8	11·5	13·2	24·8
70–79	–	2·5	2·4	3·4	4·4	7·8
> 80	1·3	3·8	5·1	0·3	1·0	1·4
Total	51·9	48·3	100·0	50·5	49·6	100·1

TABLE 2. Quantity of topical steroid and type of occlusion used

			Patients using occlusion		
Topical Steroid Usage (g/week)	Patients No.	Patients %	No. using polythene	No. using impregnated bandages	Total %
≯ 15	158	53·9	20	18	12·9
16–30	80	26·8	12	5	5·8
31–60	41	13·9	3	9	4·1
61–100	10	3·4	—	1	0·3
101–200	4	1·4	—	—	—
201–500	2	0·6	1	—	0·3
Total	295	100·0	36	33	23·4

PLASMA CORTICOSTEROIDS

Time of collection

In the steroid-treated group, blood was taken for plasma corticosteroid estimation from 34·5% of the patients between 9.45 and 11.00 a.m. and from 52·2% between 1.45 and 3.45 p.m. The proportions for the control patients were 25·9 and 58·9% respectively. The difference between the means of the plasma corticosteroid values of patients whose blood was taken in the morning and those whose blood was taken in the afternoon was just significant in both groups (Control patients $t = 1{\cdot}97$, $P = 0{\cdot}05$.

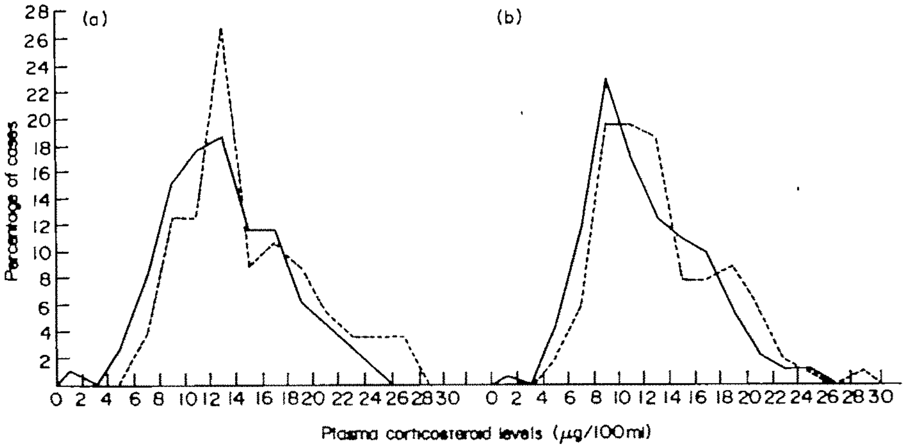

FIGURE 1. Comparison of (a) morning and (b) afternoon plasma corticosteroid distributions for topical steroid treated and control out-patients. (a) - - - - -, untreated controls, $n = 56$; ———, out-patients treated with halogenated topical steroid, $n = 113$. (b) - - - - -, untreated controls, $n = 102$; ———, out-patients treated with halogenated topical steroids, $n = 182$.

Treated patients $t = 2{\cdot}1$, $P<0{\cdot}05$). We therefore compared morning and afternoon values separately for the control and treated groups.

Corticosteroid levels

The mean plasma corticosteroid level in the patients treated with topical steroid and sampled in the morning was 13·2 μg/100 ml (SD 4·5) compared with 15·0 μg/100 ml (SD 5·0) for untreated controls. The mean plasma corticosteroid level in patients treated with topical steroid and sampled in the afternoon was 12·0 μg/100 ml (SD 4·4) compared with 13·4 μg/100 ml (SD 4·7) for untreated controls. The differences between these two sets of means is significant ($P<0{\cdot}02$ in both cases). Figure 1 illustrates

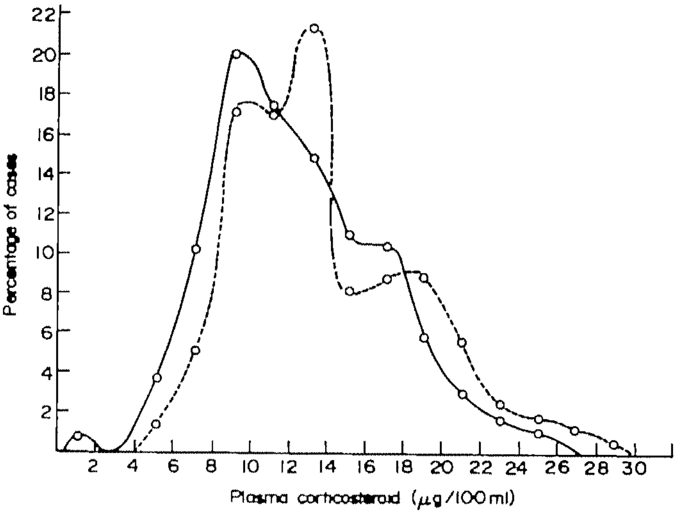

FIGURE 2. Distribution of plasma corticosteroid values of out-patients. ———, Out-patients treated with halogenated topical steroid (total no. 295); - - -, untreated control out-patients (total no. 158).

the distribution of the plasma corticosteroid values for both groups. Combining morning and afternoon samples gives a mean corticosteroid value of 12·5 µg/100 ml for the treated patients compared with 14·0 µg/100 ml for the untreated controls. The distribution of treated and control patients' corticosteroid values is shown in Fig. 2.

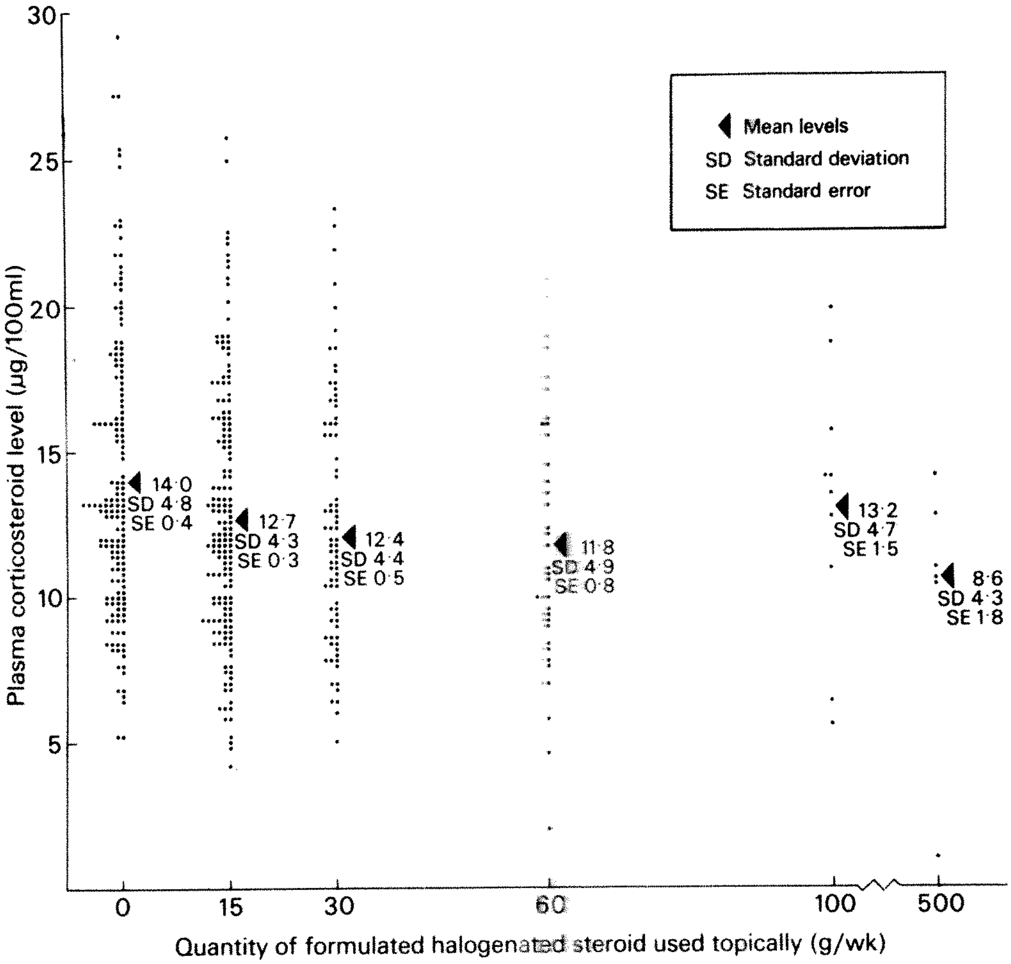

FIGURE 3. Distribution of plasma coricosteroid levels related to the quantity of topical steroid used.

Levels in relation to topical steroid usage

Figure 3 illustrates the relationship between the plasma corticosteroid values of patients and their weekly usage of topical steroid. Six of the 295 patients in the treated group had plasma corticosteroid values of less than 5 µg/100 ml. Each of these patients subsequently had a second estimation performed and the results are shown in Table 3.

DISCUSSION

Any attempt to study patients under the conditions of ordinary out-patient treatment, rather than

TABLE 3. Repeat plasma corticosteroid levels in patients with < 5 mg/100 ml

No.	Sex	Approximate area treated (%)	Initial treatment		Plasma corticosteroid level (μg/100 ml) (time)	Repeat plasma corticosteroid level (μg/100 ml) (time)	Time interval (weeks)	Changes in treatment
			Steroid used (g/week)	Occlusion				
1	Male	55 Limbs and shoulders	50	Impregnated bandages to arms and legs	2·0 (a.m.)	9·2 (p.m.)	2	Slightly less steroid used. No occlusion
2	Male	90 All over	500	None	1·0 (p.m.)	2·2 (a.m.)	5	400 g/week steroid used
3	Male	20 Face and legs	6	None	4·9 (p.m.)	11·1 (p.m.)	3	No change
4	Female	35 Thighs and arms	5	None	4·7 (a.m.)	11·9 (p.m.)	12	No steroid treatment for 12 weeks
5	Female	60 All over	60	None	4·5 (p.m.)	7·5 (p.m.)	4	50 g/week steroid used
6	Female	20 All over	3	None	4·1 (p.m.)	11·9 (p.m.)	2	7 g/week steroid used

those prevailing for hospital in-patients, has inherent disadvantages. Especially was it realized that only limited reliance could be placed on single estimations of plasma corticosteroid. By studying a large group of topical steroid treated patients and controls we have attempted to minimize this limitation.

In two reasonably comparable groups of patients those who were being treated with halogenated topical corticosteroids tended to have lower plasma corticosteroid levels than those who were not. Ninety-six percent of the treated group, compared with 99% of the control group, had plasma corticosteroid levels of >6 μg/100 ml—the generally accepted lower limit of normal (Cope, 1964).

As would be expected, the usage of topical steroids varied considerably. Over half of the 295 patients used 15 g or less per week. On the other hand two patients were using between 200 and 500 g weekly. There was a tendency for the patients who were using the highest amounts of steroid applications to have the lowest plasma corticosteroid levels, although this was not always the case.

We could find no correlation between plasma corticosteroid levels and the duration of treatment with topical steroids. The skin diseases for which this type of treatment is frequently used are characterized by exacerbations and remissions, with a consequently varying rate of topical steroid usage. In addition, the degree of absorption through the skin may vary according to the degree of inflammation present. Scoggins & Kliman (1965) showed that triamcinolone cream applied to an arm with extensive dermatitis caused a reduction in plasma corticosteroid whereas when, after the levels had returned to normal, the same quantity of steroid was applied to the patients' other unaffected arm no systemic effect could be detected. And James *et al.* (1967) noted that, in two patients treated with betamethasone 17-valerate, plasma corticosteroid and urinary 17-OH steroid levels tended to increase during the last few days of treatment. Thus, it is likely that if adrenal suppression does occur it will be transient and plasma corticosteroid levels will return to normal as the acute condition resolves, despite continued therapy. This seems the most likely explanation for the normal values found in patients 3, 5 and 6 (Table 3) when their plasma corticosteroid was measured on a second occasion.

Sixty-nine patients used some type of occlusive dressing. Only one of this sub-group was among the six patients with corticosteroid levels less than 5 μg/100 ml (patient No. 1 Table 3). When this patient's plasma level was re-assayed 2 weeks later, when Quinaband bandages were no longer being used but the weekly steroid usage was much the same, a normal value was recorded. The mean plasma corticosteroid level of the sub-group that used occlusive dressings was not significantly different from the mean of the group as a whole ($P > 0{\cdot}1$).

CONCLUSION

Unless prescribing habits in other hospital skin clinics differ markedly from those in the department we studied, it appears unlikely that more than a very small proportion of patients being treated with halogenated topical steroids have plasma corticosteroids levels below normal limits. Occlusive dressings are known to increase percutaneous absorption but their use at night and for circumscribed areas such as the hands or feet, does not appear to result in an increased risk of adrenal suppression. When plasma corticosteroid levels are low as the result of the use of topical steroids the likelihood is that values will return to normal as the skin heals, even while topical applications continue.

All the patients we studied were over 10 years of age. There is some evidence (Feiwel, Munro & James, 1967; Feiwel, James & Barnett, 1969) that children, perhaps because of their greater ratio of surface area to body weight, may more readily absorb steroids through the skin. Further studies are needed in that special group of patients.

ACKNOWLEDGMENTS

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Pituitary–adrenal function after prolonged use of topical corticosteroids

D. D. MUNRO AND D. C. CLIFT

Department of Dermatology, St Bartholomew's Hospital, London, E.C.1., and Edgware General Hospital, Middlesex

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SUMMARY

Forty patients with chronic skin disease who have used potent topical corticosteroids for a prolonged period were studied to determine if adrenal axis suppression had occurred. Thirty-seven cases (92·5%) were normal when the first test was performed and all were normal when retested. Adrenal axis suppression was therefore not of importance in this group of typical outpatients with chronic skin disease. This is an important finding to put into perspective a theoretical rather than a real complication of topical corticosteroid therapy in adults.

Absorption of topically applied corticosteroids occurs in normal individuals but especially when the skin is diseased and the integrity of the horny layer of the epidermis is disrupted. The use of occlusive dressings on the skin after topical preparations have been used, greatly enhances penetration, due to increased temperature and humidity of the skin. Many publications demonstrate that virtually all steroid compounds will penetrate the skin, and if enough is absorbed and the compound is potent enough, adrenal axis suppression will occur (Scoggins, 1962; Scholtz & Nelson 1965; Scoggins & Kliman 1965; Munro, Feiwel & James, 1967; James, Munro & Feiwel, 1967).

The significance of adrenal axis suppression in relationship to prolonged use of topical corticosteroids by patients suffering from chronic skin disease, has not been adequately investigated. This paper reports a project undertaken to determine whether suppression occurs in patients using corticosteroid topically for control of their skin diseases, as outpatients.

METHODS AND MATERIALS

Forty patients who suffered from eczema or psoriasis and attended the out-patient clinic were studied. Insulin stress tests, as described by Greenwood, Landon & Stamp (1966), were used to determine if adrenal axis suppression was present. The patients were selected for their prolonged use of topical corticosteroids. If systemic corticosteroids or corticotrophin had been administered during the 6 month period prior to the test, they were excluded from the series. The patients were fasted for 9 h before the test which was always conducted at 9.00 a.m., they were advised to continue using corticosteroid preparations to their skin as usual on the morning of the test. The patients rested on beds in the day ward, and an intravenous cannula was inserted at least 15 min before the initial resting blood samples were withdrawn. Soluble insulin (0·15 units/kg body weight) was administered intravenously, and blood sugar and plasma 11–OHCS levels were estimated from samples taken during the 2 h interval after the insulin had been given.

The personal details of the patients were recorded including their disease duration and surface involved, amount and type of topical steroid used and whether they had occluded their skin with polyethylene film.

Insulin stress tests were repeated after an interval in those cases in whom the first test was abnormal.

RESULTS

The group of forty patients consisted of seventeen women (42·5%) and twenty-three men (57·5%); their ages ranged from 15 to 80 years. Details are shown in Table 1.

A composite table (Table 2, column A) shows the proportion of body surface involved by eczema or psoriasis using the 'rule of nine' to make the calculation. Twenty-one patients (52·5%) had under 20% of their skin diseased.

TABLE 1. Sex and age distribution of the patients

Age	% Men	% Women	% Total
10–19	5	5	10
20–29	15	–	15
30–39	7·5	2·5	10
40–49	10	15	25
50–59	10	12·5	22·5
60–69	7·5	7·5	15
70 and over	2·5	–	2·5
Total	57·5	42·5	100

The steroid applied (Table 2, column B) is expressed as grams of ointment or cream per week. Twenty-two of the patients used 0·1% betamethasone 17-valerate ointment alone, thirty patients used either this preparation alone or on one part of the body, while at another site used 0·025% fluocinolone acetonide. The other ten patients used a variety of corticosteroid ointments including these two and in addition 0·025% beclomethasone dipropionate and small amounts of 1% hydrocortisone acetate ointment.

The duration of treatment (Table 2, column C) expressed in months shows that thirty-one patients (77·5%) had received therapy for 3–6 years.

Polyethylene film had been used for occluding the skin in twenty patients (50%) but most had used polyethylene gloves only, or covered relatively small areas of their skin.

The criterion used for determining a normal insulin stress response was a rise in plasma 11–OHCS to 20 μg/100 ml and an increment of 7·5 μg/100 ml above the lowest level, adequate stressing being obtained in all cases by the blood sugar falling to below 40 mg/100 ml.

Of the forty patients studied thirty-seven (92·5%) had a normal response on first testing (Fig. 1). When the tests were repeated in the three cases with initial abnormal results after 2–5 months with the patients using half their previous dose of topical corticosteroid ointment, all the patients had essentially normal results (one was minimally below the normal range with a maximal level of 19·5 μg/100 ml and an increment of 12 μg/100 ml).

TABLE 2. Proportion of diseased skin, weekly dose of applied steroid ointment and duration of therapy

	A Percentage of skin surface involved		B Steroid ointment applied (g/week)		C Duration of treatment-(months)	
	Patients		Patients		Patients	
*	No.	%	No.	%	No.	%
less than 10	14	35	11	27·5	2	5
10–19	7	17·5	13	32·5	3	7·5
20–29	4	10	6	15	4	10
30–39	4	10	6	15	9	22·5
40–49	3	7·5	–	–	4	10
50–59	4	10	–	–	–	–
60–69	2	5	1	2·5	7	17·5
70–79	–	–	–	–	4	10
80–89	–	–	–	–	–	–
90–99	2	5	1	2·5	3	7·5
100 and over	–	–	2	5	4	10
Total	40	100	40	100	40	100

* The figures in this column refer in turn to: percentages for column A, grams of ointment for column B, duration in months for column C.

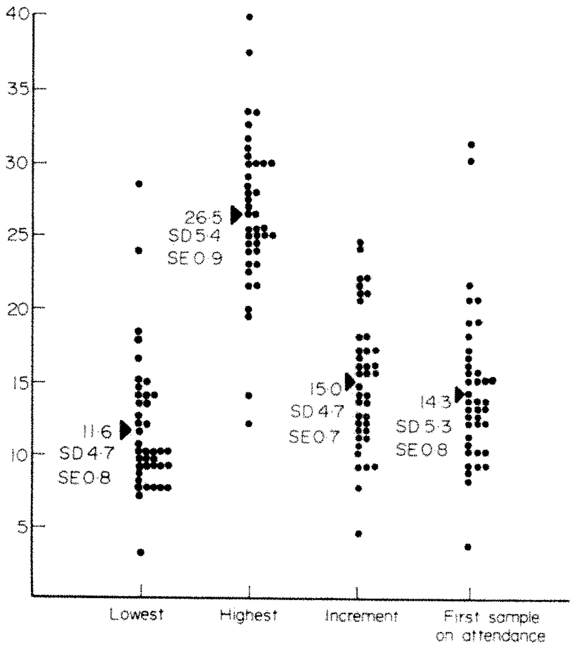

FIGURE 1. Results of insulin stress tests. A normal response is a rise to 20 μg/100 ml and an increment above the lowest level of 7·5 μg/100 ml. Arrow head denotes mean; SD, standard deviation; SE, standard error.

In the statistical assessment of the results a figure for 'dose' was determined for each case by multiplying the weekly amount of steroid ointment applied, duration of therapy and surface area of diseased skin. This 'dose' was compared with the highest, lowest, and increment figures for 11-OHCS, and no correlation was demonstrated statistically between the figures.

In order to compare the results of this investigation with that of Wilson, Williams & Marsh (1973) the first control plasma 11-OHCS sample taken during the test was recorded (Fig. 1). A reading of 8 μg/100 ml or above was obtained in thirty-nine patients (97·5%), the single case with a level of 3·5 μg/100 ml was one of the patients with an abnormal stress response.

DISCUSSION

When this project was initially considered many factors seemed to militate against producing satisfactory results. These included the variable nature of the skin diseases being studied, their tendency to remit and relapse, the variable quantity and type of corticosteroid preparation used and the inaccuracy of recording areas of diseased skin. The results of the investigation, however, have indicated that the vast majority showed no evidence of impaired adrenal axis response to insulin.

The three cases in whom the response was abnormal initially were using 25, 30 and 100 g of betamethasone ointment weekly, and the first two patients used polyethylene film occlusion on large areas of their body at times over a 10 year and 2 year period respectively, when the disease was troublesome. The third patient was a very small lady in whom 100 g of ointment weekly represented an especially large dose, and she had used this therapy for 3 years. When these cases were persuaded to use about half of the initial dose of steroid ointment their disease was equally well controlled and the repeated tests were normal in two and essentially normal in the third case.

The complications of topical corticosteroids have been discussed at length in various papers (Thorn, 1970; Munro, 1970; Macdonald, 1971). Amongst the complications usually mentioned, suppression of the adrenal axis is commonly given prominence and tends to be overstated in terms of risk to the patient. In infants and small children this may be important (Feiwel *et al.*, 1967) but in the majority of adults who use small quantities of ointments without polyethylene occlusion, there is negligible risk. The risk is increased by widespread skin disease and intensive treatment with occlusion. Other complications, such as atrophy of the skin, are much more important.

Full assessment of the hypothalamic–pituitary–adrenal axis cannot be undertaken by the use of a single test, but the insulin stress test is particularly useful to determine the integrity of the whole axis. Single plasma 11-OHCS readings give limited information but it is interesting to note that in a recent project Wilson *et al.* (1973) studied patients, who had been treated with topical corticosteroid, by random plasma 11-OHCS estimations and showed that 96% had levels of 6 μg/100 ml or above. In the present study thirty-nine patients (97·5%) had initial readings above 6 μg/100 ml and two of the cases with abnormal insulin stress tests would have been considered normal by these workers and by Cope (1964).

The purpose of this investigation was to show that patients with chronic skin disease using the quantity of corticosteroid ointments commonly prescribed in general practice and hospital out-patient clinics, are not significantly at risk from adrenal axis suppression, and this has been demonstrated.

ACKNOWLEDGMENTS

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Case Reports

Relapsing Polychondritis

N.R.ROWELL AND J.A.COTTERILL

University Department of Dermatology, The General Infirmary, Leeds

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SUMMARY

Relapsing polychondritis was first described by Jaksch-Wartenhorst in 1923. The disorder is characterized by recurrent inflammation of the cartilaginous tissue of the ears and nose, and less frequently of the ribs, larynx and tracheal rings, in association with fever, arthropathy, involvement of the eyes and an elevated erythrocyte sedimentation rate and other laboratory abnormalities. Whilst over 110 cases have been reported, mostly in the American and European literature, only six case reports have appeared in the British literature (Davies & Kelsall, 1961; Thould, Stansfeld & Balme, 1965; Hewer, 1967; Hughes *et al.*, 1972).

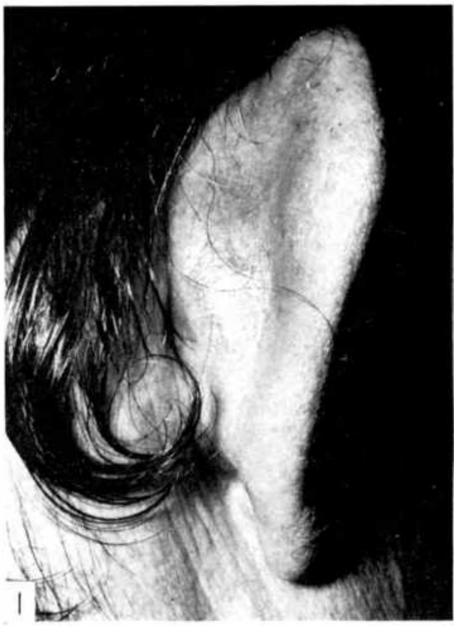
We report a further patient who showed not only the cardinal features of this unusual disease but also some hitherto unreported manifestations.

CASE REPORT

The patient, a white male aged 49 years, was first seen in January 1971, with an 18-month history of painful red hot swellings of the feet and tenderness over superficial veins of the legs, responding to prednisone given empirically in a dose of 30 mg daily. Cessation of this therapy was rapidly followed by clinical relapse and systemic antibiotic therapy did not control the disease. In June 1970, the patient developed a painful red swollen right ear, with a temperature of 39°C. Ibuprofen was prescribed but this drug was stopped when the patient developed a toxic erythema. Several episodes, tentatively diagnosed as erysipelas of both ears, occurred subsequently, for which the patient received cloxacillin. In October 1971, recurrent episodes of superficial thrombophlebitis, mostly in the legs but also to a lesser extent in the arms after minor trauma, occurred. Phenylbutazone therapy was helpful at this time, whilst therapy with phenformin and ethylestrenol proved ineffective.

In December 1971, a severe exacerbation of the illness occurred with an extensive toxic erythema, pain and swelling of knees, ankles and wrists, tenosynovitis at both wrists, thrombophlebitis of the veins in the left antecubital fossa and exquisite tenderness of the costochondral cartilages. The episode settled in 1 week without therapy but in January 1972, the patient's nose became acutely red, tender and swollen and his ears were noted to be floppy and atrophic (Fig. 1). In March 1972, the patient developed pain and swelling of the thyroid cartilage. At this stage, prednisone 10 mg t.d.s. was prescribed and he is now almost symptom free on enteric-coated prednisolone 10 mg daily.

In subsequent exacerbations the cartilaginous parts of the ears have been red, swollen, firm and tender and sparing of the ear lobes has distinguished the condition from erysipelas (Fig. 2). Between inflammatory episodes the ears are soft and tend to hang forward (Fig. 1).



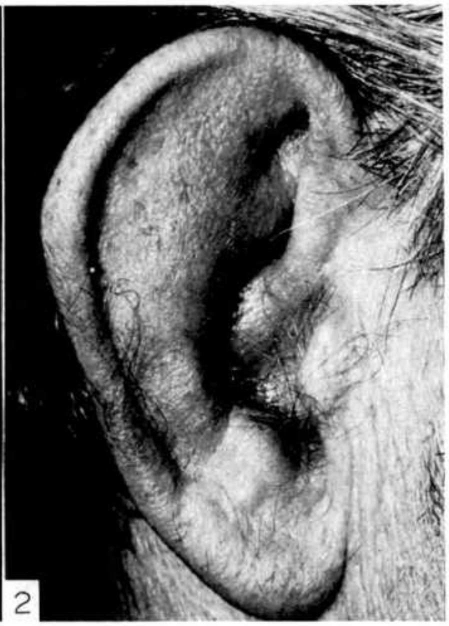


FIGURE 1. Showing an atrophic ear tending to flop forwards.

FIGURE 2. Showing inflammation over the cartilaginous part of the ear with sparing of the lobulus.

Other salient features have been pain, tenderness, swelling and redness of the nose in the region of the nasal cartilage and painful swollen wrists, ankles and knees. The thyroid cartilage became acutely tender and inflamed on one occasion. There were no abnormal findings in the cardiovascular system and no lymphadenopathy. His eyes and hearing were normal.

Investigations

Hb 11·3–12·1 g/100 ml; WBC 7700/mm^3—normal differential. Platelets normal; reticulocytes 1·1–4·9%; MCV 102–106 μm^3; MCHC 32·8%; ESR 27–100 mm in 1 h; serum folate 2·4–4·4 μg/ml; red cell folate 160 μg/ml; serum B$_{12}$ 450 and 835 pg/ml. Bone marrow examination revealed an active marrow with marked granulocytic hypoplasia. Coombs' test negative; LE cells not detected; haptoglobins normal; methaemalbumin not detected. Red cell survival was normal at 63 days. Euglobin lysis time 414 min (normal $<$230 min). Tissue fibrinolysis (Cunliffe *et al.*, 1971) of both involved and non-involved leg skin was grossly reduced ($<$2·5%). Blood urea, electrolytes and creatinine clearance normal. Liver function tests and glucose tolerance test normal. Total serum proteins 8·0 g/100 ml, albumin 4·4 g/100 ml, globulin 3·6 g/100 ml. IgG 1250 mg/100 ml (normal range 700–1500 mg/100 ml), IgA 565 mg/100 ml (normal range 60–490 mg/100 ml), IgM 270 mg/100 ml (normal range 37–204 mg/100 ml). RA latex test strongly positive but differential agglutinin test negative; ASO titre 1 in 200 and 1 in 50. Anticartilage antibodies (Dolan, Lemmon & Teitelbaum, 1966) not detected. Gastric parietal cell antibodies—weak positive; thyroid microsomal antibodies positive; antinuclear factor negative. Urinary acid mucopolysaccharide hexosamine 11 mg/24 h (normal range 3·0–7·5 mg/24 h). Chest X-ray normal. Tracheal tomograms, soft tissue pinnograms and nasograms normal. Skeletal survey and joints radiologically normal. ECG normal.

Histological examination of an erythematous papule taken from the leg showed no convincing evidence of vasculitis.

DISCUSSION

The most characteristic feature of relapsing polychondritis is inflammation of cartilage. Over 110 cases have now been reported in the world literature and the subject has been reviewed by several authors (Bean, Drevets & Chapman, 1958; Kaye & Sones, 1964; Dolan *et al.*, 1966, Hughes *et al.*, 1972). Our patient showed the classical clinical features of this disease including recurrent inflammation of ear, nasal and rib cartilages, arthritis and, finally, involvement of the larynx. He also had a raised ESR and an anaemia with a variable reticulocytosis which are common findings in relapsing polychondritis. The cause of this anaemia in our patient remains obscure as there was no laboratory evidence for a haemolytic process and the bone marrow appeared active. Moreover, the plasma volume was normal as were the serum iron, B_{12} and folates. Our patient also showed a raised urinary acid mucopolysaccharide excretion during an exacerbation. Kay & Sones (1964) have demonstrated that this is a useful laboratory investigation in relapsing polychondritis during an acute episode.

The clinical manifestations of relapsing polychondritis are so variable that patients with this disorder may be seen by clinicians in many widely differing specialties. The dermatologist, however, is especially likely to see such cases as the commonest manifestation is recurrent acute swelling with erythema and tenderness of the pinna of the ear. It is important to notice that the lobulus is spared in this inflammatory process (Fig. 2) and that this finding may serve to distinguish this condition from recurrent erysipelas, which is the commonest early misdiagnosis. With progression the ears become atrophic and floppy (Fig. 1).

Other dermatological manifestations include inflammation over the nasal cartilage which may lead to a saddle nose deformity, and erythematous swelling around inflamed joints. Other skin lesions reported include alopecia (Hilding, 1952; Bean *et al.*, 1958), retarded nail growth (Bean *et al.*, 1958) and erythematous nodules (Winer & Levin, 1960; Pearson, Kline & Newcomer, 1960). Our patient presented with such lesions. Psoriasis proven by biopsy has been described (Pearson *et al.*, 1960) but this is almost certainly a chance association. Hyperpigmentation over involved cartilage has also been reported (Arundell & Haserick, 1960; Dolan *et al.*, 1966).

Our patient had two episodes of toxic erythema and recurrent thrombophlebitis. This latter finding has not been previously described in relapsing polychondritis. However, several case reports in which a vasculitis was thought to be a significant feature in the pathogenesis of relapsing polychondritis have appeared (e.g. Anderson, 1966; Lamy *et al.*, 1967; Christianson, Weiss & Kilpatrick, 1969; Hughes *et al.*, 1972).

Although the biopsy of an active erythematous papule did not show any frank evidence of vasculitis histologically, the tissue fibrinolytic activity of this lesion was significantly reduced. The euglobin lysis time was also much prolonged. Such a decrease in blood fibrinolysis is more likely to be due to secondary thrombosis rather than to primary phenomena (Cunliffe *et al.*, 1971) and it is known that a decrease in skin fibrinolytic activity occurs both in patients with vasculitis (Cunliffe *et al.*, 1971) and in patients with thrombophlebitis (Pandolfi, Isacson & Nilsson, 1968). It would seem that a vasculitis may have been responsible for some of the clinical features in our case.

The aetiology of relapsing polychondritis remains obscure but it is interesting that our patient had gastric and thyroid antibodies and a positive RA latex test. Relapsing polychondritis has been described in association with autoimmune thyroid disease (Goldwater, 1963; Kaye & Sones, 1964) and rheumatoid arthritis (Pearson *et al.*, 1960; Kaye & Sones, 1964). Other associations with autoaggressive diseases include Sjögren's syndrome (Rogers & Lansbury, 1955; Coste *et al.*, 1961), lupus erythematosus (Kaye & Sones, 1964; Marshall & Le Rouse, 1964) and systemic sclerosis (Middleton, 1962). We were unable to detect anticartilage anti-bodies by the immunofluorescent method of Dolan *et al.* (1966).

The satisfactory response to corticosteroids in most patients lends further support to the concept of an autoaggressive aetiology. Our patient responded well to oral prednisone and remains symptom free on a small maintenance dose but if this dose is reduced a further exacerbation promptly occurs.

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Recurrent Kaposi's varicelliform eruption in Darier's disease

P.G. HIGGINS* AND K.D. CROW

Virus Laboratory of the Public Health Laboratory Service, Cirencester, and Princess Margaret Hospital, Swindon, Wilts.

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SUMMARY

This paper describes five attacks of Kaposi's varicelliform eruption which occurred during a period of 18 months in a woman with Darier's disease. Herpes simplex virus type 1 was isolated from the lesions in three of the four episodes which were studied in the laboratory and Coxsackievirus type A16 from the remaining attack. It is suggested that Coxsackievirus A16 may be a hitherto overlooked cause of Kaposi's varicelliform eruption.

Infection with any one of a number of viruses may be accompanied by vesicular lesions of the skin. Herpes simplex virus and vaccinia virus are the two most frequently found affecting established skin disease, especially atopic eczema, when the term Kaposi's varicelliform eruption is commonly used to describe the condition. Hyperkeratosis follicularis, Darier's disease, is an uncommon dyskeratosis with a characteristic clinical and histological picture and may be complicated by secondary viral infection. In most of the published reports of Kaposi's varicelliform eruption in Darier's disease, herpes simplex virus has either been assumed to be the infecting agent (Bleiberg & Klosk, 1951; Kesten, 1952; Hitselberger & Burns, 1961) or actually proven to be by virus isolation (von Weiss, Kibrick & Lever, 1965; Doeglas & Moolhuysen, 1969; Izumi & Goldschmidt, 1970). Infection with vaccinia virus has also been recorded (Gerstein & Shelley, 1960).

The purpose of this paper is to report the occurrence of five attacks of Kaposi's varicelliform eruption in a patient with Darier's disease within a period of 18 months, together with the virological findings.

CASE REPORT

The patient was a woman of 31 years when she first came under the care of one of us (K.D.C.) in 1965. At this time she gave a 22-year history of classical Darier's disease which was generalized affecting the whole skin, but less marked on the face. The ulceration and pain of lesions in the perineum was such that skin grafting in this region was considered but was decided against because of the lack of suitable donor sites.

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* Present address: Virus Reference Laboratory, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT.

Skin biopsy showed the typical features of hyperkeratosis follicularis with numerous corps ronds, grains and lacunae.

The patient was reticent to give details of other members of her family, but it is known that at least one sibling is affected by Darier's disease.

In January 1970 she was admitted to the Princess Margaret Hospital, Swindon, gravely ill, temperature 103°F and with a generalized vesicular eruption over the whole of the trunk, neck and proximal part of the limbs involving both normal and hyperkeratotic skin. Clinically she was considered to be suffering from a primary infection with herpes simplex virus and was treated with cytosine arabinoside 10 mg intravenously daily for 5 days. There was a dramatic improvement and the patient appeared to be out of danger within 24 h and many of the lesions were crusting in 48 h. By the fifth day of treatment all lesions had dried to leave brown crusts and no fresh vesicles had developed for 48 h. The patient

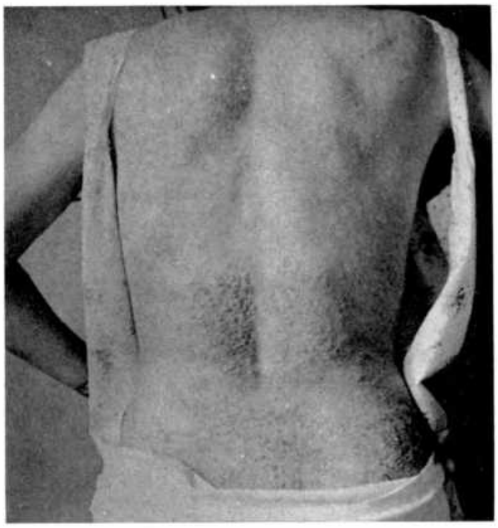

FIGURE 1. Distribution of the lesions of the fifth episode of Kaposi's varicelliform eruption.

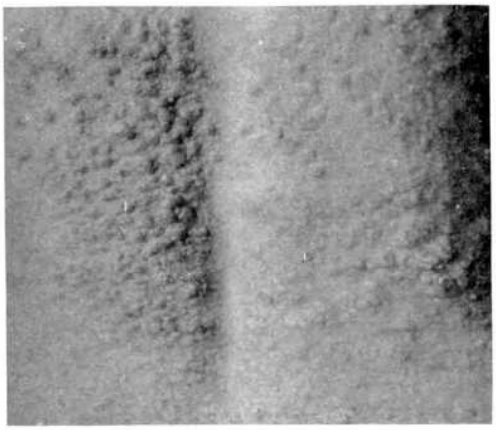

FIGURE 2. Typical appearance of the lesions seen in the last four episodes of Kaposi's varicelliform eruption.

left hospital 13 days after admission. When seen 2 weeks later those areas affected by the herpes simplex virus were clear of Darier's disease which subsequently reappeared.

The second attack of Kaposi's varicelliform eruption commenced in mid-March 1970, but was not accompanied by the severe constitutional disturbance that was observed in the first attack and the lesions were limited to the back of the trunk. The illness subsided without specific treatment over 11 days.

Three further attacks followed in June 1970, November 1970 and July 1971. The illnesses were similar to the second attack and the vesicular eruption was identical on each occasion (see Figs. 1 and 2). No lasting improvement in the patient's hyperkeratosis follicularis was observed and no further episodes occurred between July 1971 and when the patient was last seen in January 1972.

VIROLOGICAL FINDINGS

Specimens for virus isolation consisted of vesicle fluid when available or scrapings and swabs from the base of the lesions. This material was inoculated into tissue cultures of monkey kidney, the Bristol line of HeLa, human embryo kidney and human embryo diploid fibroblasts (WI-38). Herpes simplex virus was isolated from specimens collected on each of the first 4 days of the initial attack but from none of 4 subsequent daily specimens which were examined. Similarly, during the second episode in March 1970 herpes simplex virus was isolated from each of the specimens collected on the first 5 days of the illness, but not from those collected on the following 3 days. Only one specimen was received during the third attack (June 1970) and this failed to yield a virus in tissue culture but inoculation of the specimen into suckling mice less than 48 h old resulted in the appearance of typical flaccid Coxsackie A type paralysis. This virus was later shown to belong to type A16. No material was received from the fourth eruption but a single specimen collected during the fifth attack in July 1971 again yielded herpes simplex virus. The strain of herpes simplex virus associatied with each of the episodes was shown to belong to type 1 as determined by its appearance on the chorioallantoic membrane of the developing chick embryo.

Neutralizing antibody to herpes simplex virus and Coxsackie A16 virus was assayed in tissue culture using 100 TCD_{50} of virus challenge. Antibody to herpes simplex virus rose from 1/8 at the onset of the first attack to 1/32 at the beginning of the second episode and to $\geqslant 1/64$ at the commencement of the third attack. Neutralizing antibody to Coxsackie virus A16 at a dilution of 1/8 was not demonstrable in any serum collected between the first and last attacks.

DISCUSSION

This patient demonstrated the classical picture of hyperkeratosis follicularis and presented many of the features previously recorded when Kaposi's varicelliform eruption is superimposed on this disease. The primary infection with herpes simplex virus, confirmed by virus isolation and serology, was accompanied by a profound constitutional disturbance as was seen in the patients observed by von Weiss and his colleagues (1965) and by Izumi & Goldschmidt (1970). The dramatic response to cytosine arabinoside confirmed the reports of the beneficial effect of this compound in herpes simplex infections (Chow, Foerster & Hryniuk, 1970; Juel-Jensen, 1970). The lesions of Darier's disease regressed following infection but reappeared later with no lasting improvement—a feature also noted by others (Bleiberg & Klosk, 1951; Gerstein & Shelley, 1960; Doeglas and Moolhuysen, 1969). Second attacks of Kaposi's varicelliform eruption in Darier's disease as a result of infection with herpes simplex virus were reported by Doeglas & Moolhuysen (1969) in two patients, but further proven attacks have not been recorded although one patient with hyperkeratosis follicularis is reported to have had seven attacks of 'herpes' (Kesten, 1952).

The most interesting finding is this case was the isolation of Coxsackievirus type A16 from vesicle fluid collected during the third attack of Kaposi's varicelliform eruption. The rash was identical to that present when herpes simplex virus was isolated. Coxsackievirus A16 is one of the serotypes capable of causing hand, foot and mouth disease and in this infection the lesions in the mouth are very difficult to distinguish from those resulting from infection with herpes simplex virus except by their distribution. The absence of antibody in patients with Coxsackievirus A16 infections has been reported previously (Robinson, Doane & Rhodes, 1958) and may result from the use of the prototype strain of A16 virus as the virus challenge in neutralization tests, for this strain has been shown to be less sensitive to antibody to some wild strains than the homologous virus (Alsop, Flewett & Forster, 1960). Alternatively it may be related to the age of the patient for Morgante and his co-workers (1972) were unable to show any neutralizing antibody in the sera from either of the two adults from whom Coxsackievirus type A16 was isolated, but demonstrated rising antibody titres in seventeen out of nineteen children with hand, foot and mouth disease in the same outbreak. The isolation of Coxsackievirus A16 from vesicle fluid in this case strongly suggests a causal relationship between infection with this virus and Kaposi's varicelliform eruption. It is thought unlikely that specimens from this condition have been examined routinely in young suckling mice and it is possible that other cases have resulted from infection with Coxsackievirus A16.

ACKNOWLEDGMENTS

We are grateful to Dr R. H. Cowdell for the report on the histology of the skin biopsy, Dr B. E. Juel-Jensen for supplies of cytosine arabinoside and to Dr D. R. Gamble for typing the Coxsackie A virus.

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Pseudosarcomatous dermatofibroma

P.B.THAVABALAN AND V.T.RATNAIKE*

Department of Dermatology, University College Hospital Medical School, University Street, London, W.C.1

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SUMMARY

A pseudosarcomatous dermatofibroma with marked collagen damage is reported. The multinucleated giant cells and proliferation of very elongated sarcomatous-like fibroblasts histologically resemble a highly malignant sarcoma.

CASE REPORT

A married white female aged 28 presented with a small flat papule in the floor of the left axilla which had been present for 5 years. She complained that it had been occasionally painful and it had slowly increased in size.

On examination the lesion was a small, round, firm dermal nodule about 1 cm in diameter. It was removed under local anaesthesia, and sent for histological examination.

Histology

The epidermis and superficial dermis were essentially normal. The cellular proliferation was present in an ill-defined but localized area of the mid- and lower dermis; it did not penetrate into the subcutaneous tissue. There were numerous spindle and stellate cells together with many multinucleated giant cells scattered throughout the affected dermis. Only a few mitotic figures were seen and the nuclei were not grossly pyknotic. The collagen surrounding this cellular proliferation was abnormal in that it appeared hazy and there was coagulation and loss of the usual fibrillar structure seen in fixed and processed preparations. This abnormal dermis at the periphery of the cellular lesion (Fig. 1) was suggestive of a degenerative process which was affecting the collagen.

DISCUSSION

Pseudosarcomatous dermatofibroma (pseudosarcomatous reticulohistiocytoma: Gordon, 1964), atypical fibroxanthoma (Reed & O'Quinn, 1971), or the nodular pseudosarcomatous fasciitis which occurs in subcutaneous tissue, is a rare condition showing multinucleated giant cells and proliferation of elongated sarcoma-like fibroblasts.

The pseudosarcomatous dermatofibroma is of importance to the dermatologist and the histopathologist because of its great histological similarity to a malignant fibrosarcomatous tumour, in spite of its benign clinical course.

Although pseudosarcomatous dermatofibroma may be mistaken for a malignant sarcoma, it is not

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* Present address: Dermatology Department, General Hospital, Colombo, Ceylon.

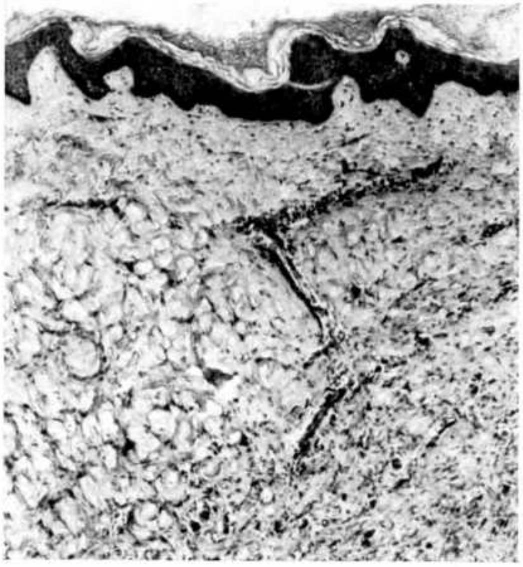

FIGURE 1. Low magnification of section showing the proliferative lesion centrally in the dermis with areas of collagen degeneration surrounding it. The lesion is not encapsulated.

known to metastasize (Levan, Hirsch & Kwong, 1963). These workers reported a series of seventeen cases, many of whom had been previously diagnosed histologically as sarcomata, but none had subsequently developed metastases although two cases had recurred locally after removal. Local recurrence is explained by the fact that the tumour is not encapsulated or well defined in its limits (Stout 1960), thus making complete removal uncertain.

A peculiar feature of pseudosarcomatous dermatofibroma is its association with degenerate collagen (Kempson & McGavran, 1964), and this was also conspicuous in our case. It is uncertain as to the relationship between this degeneration and the fibrous reaction. In lesions which are thought to be primarily due to collagen damage such as necrobiosis lipoidica and granuloma annulare, the collagen damage is central and the tissue reaction at the periphery. In this case the damaged collagen was peripheral to the fibrous reaction, and it may be that the damage was secondary and was due to the release of collagenases and other proteolytic enzymes by the highly active and proliferating fibroblasts.

ACKNOWLEDGMENTS

We wish to thank Dr A. Jarrett, for valuable help with the histology and for encouragement in reporting this case. We also thank Dr T. W. E. Robinson, for allowing us to study his case.

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Comment

COMPARATIVE PHYSIOLOGY OF SWEATING

INTRODUCTION

In the following short review an attempt has been made to collate recent work on the sweat glands of different species. Information on these glands is, however, limited and it has been necessary to generalize from sparse evidence; most of the physiological information, for example, has been obtained from study of domestic and only a few wild animals. It is hoped, however, that, in spite of the dangers of generalization, by presenting the available data in comparative terms it will provoke discussion and a challenging of present views.

Sweat glands are present only in the skin of mammals. They have been demonstrated throughout the skin of the general body surface and in specialized regions, such as the eyelids, of monotremes, marsupials and some but not all eutheria (Fig. 1). In some species, e.g. the rodents they are present only in specialized regions of the body such as the footpads and chin (Munger, 1971; Lyne *et al.*, 1964). The sweat glands of the body surface of man, and the mole (Giacometti & Machida, 1965) are not associated with hair follicles and may, therefore, be termed atrichial (Bligh, 1967). The plantar sweat glands of the Rodentia, Felidae and Canidae also fall into this category. The sweat glands of almost all other mammals are epitrichial, i.e. they are situated beside a hair follicle as part of a hair follicle unit (Jenkinson, 1965). Some primates, however, have both atrichial and epitrichial sweat glands in different proportions in their body surface skin (Montagna & Yun, 1963; Montagna, Yun & Machida, 1964; Montagna *et al.*, 1962). All hair follicles do not have an associated sweat gland; in animals such as the dog, sheep and camel which exhibit hair follicle grouping (Carter, 1955; Jenkinson, 1965) they are associated only with the primary hairs of the group. Consequently animals with a dense hair coat do not always have a high density of sweat glands. Sweat gland density varies widely between species from about 20–30/cm^2 skin in the pig to over 2000/cm^2 in Zebu cattle.

Sweat glands consist of a fundus and a duct. The latter, which is generally straight or slightly convoluted, is lined with a cubical epithelium and in the case of the atrichial glands opens directly on to the skin surface. The duct of the epitrichial gland on the other hand opens into the pilosebaceous canal more proximal to the skin surface than that of the sebaceous gland except in phlocidae (Ling, 1965) and consequently the sweat is mixed with sebum before it reaches the skin surface. The spiral portion of duct found in the relatively thick epidermis of man is not characteristic of mammals in general. The fundus of the sweat gland varies in appearance between species and within species, from a simple sac to a narrow highly convoluted or coiled tube. The glands in specialized body regions generally appear to be longer and more convoluted than those of the body surface. The fundus of all species studied consists of two layers of epithelium, (1) an inner secretory epithelium, the cells of which are flat in some species (Findlay & Jenkinson, 1964) and columnar in others, e.g. the human axilla, and (2) an outer myoepithelium composed of canoe-shaped cells (Tucker, 1968; Jenkinson, 1971).

PURPOSE OF SWEAT GLANDS

Sweat glands are present only in homeothermic animals. The homeotherms which are devoid of

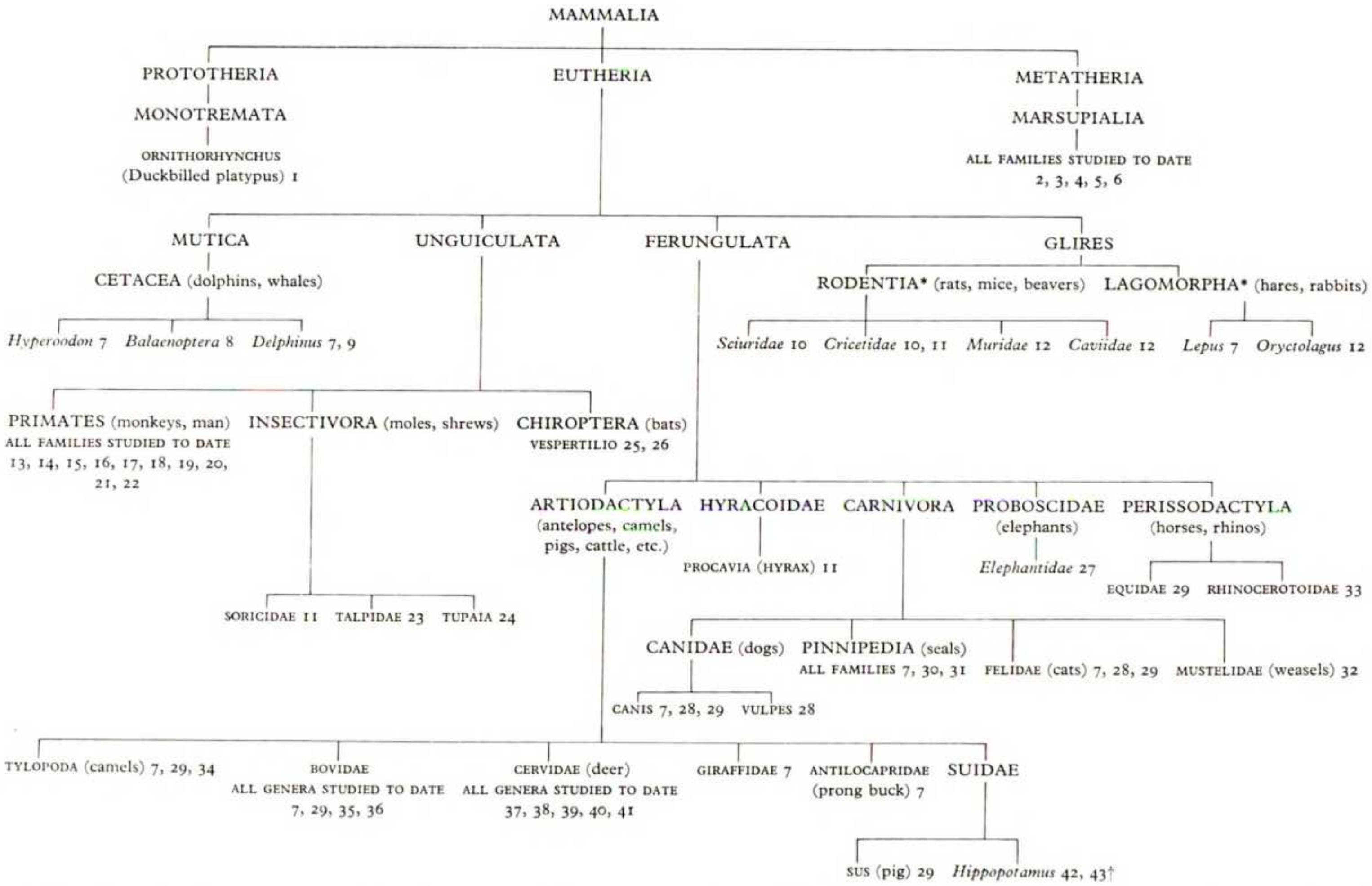
MAMMALIA
PROTOTHERIA
MONOTREMATA
ORNITHORHYNCHUS (Duckbilled platypus) 1
EUTHERIA
METATHERIA
MARSUPIALIA
ALL FAMILIES STUDIED TO DATE 2, 3, 4, 5, 6
MUTICA
CETACEA (dolphins, whales)
Hyperoodon 7
Balaenoptera 8
Delphinus 7, 9
UNGUICULATA
FERUNGULATA
GLIRES
RODENTIA* (rats, mice, beavers)
LAGOMORPHA* (hares, rabbits)
Sciuridae 10
Cricetidae 10, 11
Muridae 12
Caviidae 12
Lepus 7
Oryctolagus 12
PRIMATES (monkeys, man) ALL FAMILIES STUDIED TO DATE 13, 14, 15, 16, 17, 18, 19, 20, 21, 22
INSECTIVORA (moles, shrews)
CHIROPTERA (bats) VESPERTILIO 25, 26
SORICIDAE 11
TALPIDAE 23
TUPAIA 24
ARTIODACTYLA (antelopes, camels, pigs, cattle, etc.)
HYRACOIDAE
PROCAVIA (HYRAX) 11
CARNIVORA
PROBOSCIDAE (elephants)
Elephantidae 27
PERISSODACTYLA (horses, rhinos)
EQUIDAE 29
RHINOCEROTOIDAE 33
CANIDAE (dogs)
CANIS 7, 28, 29
VULPES 28
PINNIPEDIA (seals) ALL FAMILIES 7, 30, 31
FELIDAE (cats) 7, 28, 29
MUSTELIDAE (weasels) 32
TYLOPODA (camels) 7, 29, 34
BOVIDAE ALL GENERA STUDIED TO DATE 7, 29, 35, 36
CERVIDAE (deer) ALL GENERA STUDIED TO DATE 37, 38, 39, 40, 41
GIRAFFIDAE 7
ANTILOCAPRIDAE (prong buck) 7
SUIDAE
SUS (pig) 29
Hippopotamus 42, 43†

FIGURE 1. A diagram illustrating the mammalian families for which references to skin structure have been traced. Some of these references are cited. Capitalletters, sweat glands over lgeneral body surface; italic letters, sweat glands absent from general body surface; *, sweat glands in specialized regions, e.g. foot pads; †, subderma acinar glands.

1. Montagna & Ellis (1960)
2. Hardy & Hall (1947)
3. Green (1961)
4. Green (1963)
5. Mykytowycz & Nay (1964)
6. Hulbert & Rose (1972)
7. Jenkinson, unpublished data
8. Giacometti (1967)
9. Palmer & Weddell (1964)
10. Sokolov (1962a)
11. Wallin (1967)
12. Jenkinson (1970)
13. Machida & Giacometti (1967)
14. Montagna & Yun (1963)
15. Montagna, Yun & Machida (1964)
16. Sakurai & Montagna (1964)
17. Montagna & Yun (1962)
18. Machida, Perkins & Montagna (1964)
19. Hanson & Montagna (1962)
20. Machida & Montagna (1964)
21. Montagna & Ellis (1959)
22. Rothman (1955)
23. Giacometti & Machida (1965)
24. Montagna *et al.* (1962)
25. Murphy (1960)
26. Cortese & Nicoll (1970)
27. Smith (1890)
28. Soriano (1964)
29. Jenkinson (1965)
30. Ling (1965)
31. Montagna & Harrison (1957)
32. Reineke, Travis & Dolnick (1962)
33. Cave & Allbrook (1959)
34. Allen & Bligh (1969)
35. Sokolov (1962b)
36. Robertshaw & Taylor (1969a)
37. Quay (1955)
38. Quay (1959)
39. Quay & Müller-Schwarze (1970)
40. Quay & Müller-Schwarze (1971)
41. Jenkinson (1972b)
42. Allbrook (1962)
43. Luck & Wright (1964)

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sweat glands are those which can generally avoid extremes of temperature by creating their own microclimate, e.g. the rodents by burrowing (Schmidt-Nielsen, 1964), the cetacea by diving and the hippopotamus by wallowing (Luck & Wright, 1964). This evidence endorses the view that sweat glands are basically thermoregulatory organs. However, their efficiency as such is markedly variable between species. Those of the pig do not respond to heat (Ingram, 1967) and no marsupials are now known to use sweating as a thermoregulatory response when at rest (Hulbert & Rose, 1972). Such a response appears to be of prime importance in only two mammalian groups, the primates (Stitt & Hardy, 1971) and ungulates. Some ungulates, however, rely much more on panting than on skin evaporation for regulation of body temperature (Finch, 1972; Jenkinson, 1972a). The pattern and magnitude of sweating in Bovidae is correlated with the size of the animal (Robertshaw & Taylor, 1969a). Sweating appears to play only a minor role in thermal cooling in the sheep and red deer (Bligh, 1967; Johnson, Maloiy & Bligh, 1972). It is apparent therefore that sweat glands are not necessarily efficient thermoregulatory organs and those in specialized regions such as the footpads, palms and eyelids are unlikely to have such a function. Apart from those of man, some primates, the equines, and some Bovidae, sweat glands appear to play only a minor role in the control of body temperature in warm conditions.

Sweat glands should perhaps therefore be considered basically to be protective organs. They may, for example, protect (1) against extremes of temperature by acting as thermoregulatory organs (2) against frictional damage in specialized regions such as the eyelids and palms (3) against accumulation of waste products by acting as excretory organs (4) against bacterial action by producing sweat which may be effective either as an antibacterial secretion or by aiding the flow of sebum across the skin surface, and (5) against extinction of the species by acting as scent glands, for example, in herding artiodactyls. It seems probable therefore that the principal purpose of a sweat gland is not necessarily the same in all species or even between body regions. In some species, such as man, they may well have more than one role.

MODE OF ACTION OF SWEAT GLANDS

Different patterns of cutaneous evaporative loss which at high ambient temperatures reflect sweating activity, have been distinguished using ventilated capsules placed on the skin surface of mammals (Fig. 2) (McLean, 1963; Bligh, 1967; Allen & Bligh, 1969; Robertshaw & Taylor, 1969a, b; Bullard, Dill & Yousef, 1970; Jenkinson, 1972a; Johnson *et al.*, 1972). Bligh (1967) postulated that these patterns can be explained in terms of the relative importance of the secretory and myoepithelial cells of the glandular fundus. He suggests that the smooth onset and sustained sweating activity characteristic of man, the camel, llama and buffalo (*Synceros caffer*) and sometimes the horse (Allen & Bligh, 1969) is due to secretory activity with little or no myoepithelial involvement. The stepwise increase in sweat output to a maximum which can be sustained at least for several hours and found in the cow, burro and eland, he attributes to myoepithelial contractions superimposed upon a secretory rate rapidly rising to a maximum. The discrete synchronous discharges of sweat found in the sheep, goat, duiker and Grants gazelle, which decrease in magnitude and frequency with time he considers as being due to myoepithelial expulsion of sweat, the secretory process being slow and uncontrolled. The sweating pattern recently observed in the red deer (Johnson *et al.*, 1972) according to this hypo-

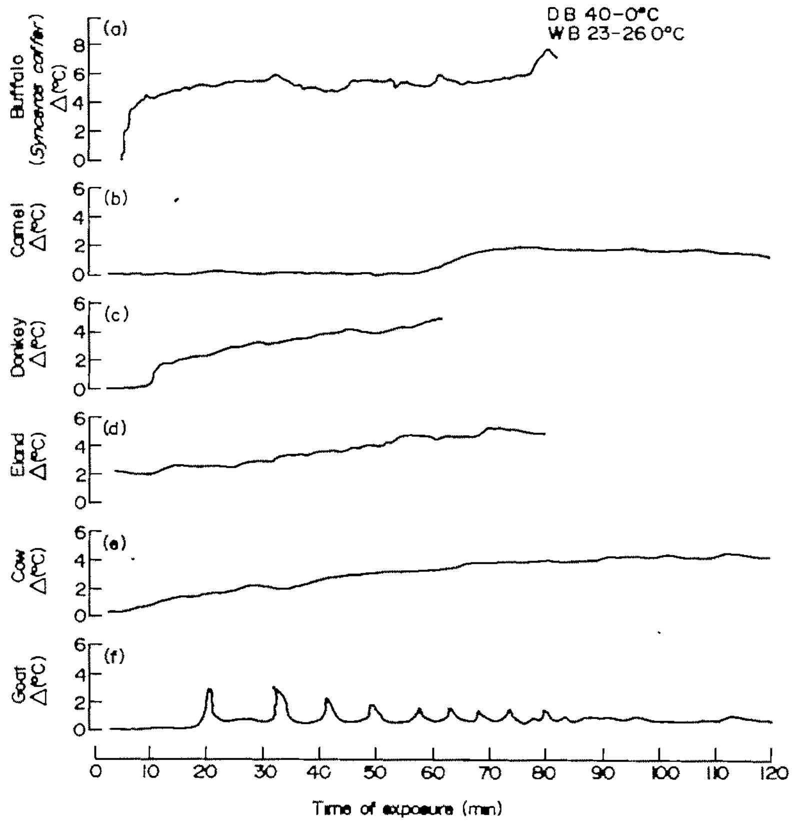

FIGURE 2. A diagram illustrating some of the different patterns of evaporative loss from the skin found in mammals. The figure has been compiled from the work of different authors. (a) Robertshaw & Taylor (1969a), (b) Jenkinson (1969), (c) Robertshaw & Taylor (1969b), (d) Robertshaw & Taylor (1969a), (e) Jenkinson (1969), (f) Jenkinson & Robertshaw (1971).

thesis would also be indicative of myoepithelial involvement in the secretory mechanism of this species.

The sparse evidence to date tends to support Bligh's hypothesis. In man, primates and the horse, glycogen is present in the secretory cells (Rothman, 1955; Smith & Dobson, 1966; Lovatt Evans, Nisbet & Ross, 1957) and is depleted after sweating, indicating an active secretory process requiring energy. In the cow which exhibits the stepwise pattern of sweating there is as yet no evidence of a potential energy store in the secretory epithelium; glycogen is not present in these cells (Yang, 1952). Recent studies of the losses of Na^+ and K^+ from cattle skin during sweating, however, indicate that either active secretion or tubular re-absorption of these electrolytes occurs and suggest the possibility of at least an osmotic mechanism (Johnson, 1970; Jenkinson & Mabon, 1973). In the goat, which exhibits the synchronous pattern of sweating, although sweat production does occur during activity, glandular fatigue is apparently due to the rate of expulsion exceeding the rate of sweat production (Jenkinson & Robertshaw, 1971). Myoepithelial action may well be of importance therefore in the expulsion of sweat in some species. Anatomical evidence (Tucker, 1968) supports this view but indicates that, since the ratio of myoepithelial cell diameter to sweat gland diameter is in general constant between species, the rate of contraction is likely to be of greater importance than the relative strength of each contraction (Jenkinson, 1971).

The comparative evidence, therefore, indicates that the rate of secretion and in view of differences in sweat composition, perhaps even the mode of secretion, varies between mammals. Since the work of Schiefferdecker (1917) sweat glands have been described as apocrine or eccrine. The epitrichial sweat glands have been described as apocrine, a nomenclature which by definition implies a specific mode of activity, viz. necrobiotic discharge. Physiological studies on the calf (Findlay & Jenkinson, 1964) have shown that such a secretory process does not occur in that species. The sweat glands of cattle and the sheep and goat do not function in the classical apocrine manner and it is doubtful if such a process of secretion exists in sweat glands (Jenkinson, 1967). Save for the axillary glands of man electron microscope studies of the secretory process in epitrichial sweat glands are lacking and the nature of the secretory mechanism or mechanisms in different species is still unknown.

SWEAT COMPOSITION

There is little known about the composition of sweat in mammals other than man and the footpads of the dog and cat. This is no doubt partly due to the technical difficulty in obtaining a pure sample of sweat since in most species a mixture of sebum and sweat emerges from the skin pores. Histochemical studies indicate that glycogen is absent from the secretory cells of some animals, e.g. the sheep, goat and cow (Yang, 1952) but is present in those of others, e.g. man, some but not all primates, the seal and horse (Rothman, 1955; Machida & Montagna, 1964; Ling, 1968; Lovatt Evans *et al.*, 1957). Lipid also is present in the glandular cells of only some species (Yang, 1952; Rothman, 1955). Electrolytes, especially Na^+, K^+ and Cl^-, have been found in the sweat of all species studied to date (McFarlane *et al.*, 1958; Johnson, 1970; Jenkinson & Mabon, 1973). In the ruminants, which have a high K^+ intake relative to man, the loss of K^+ in sweat exceeds that of Na^+ in warm conditions in contrast to human sweat which normally has a high Na^+/K^+ ratio (Collins, 1962). There is insufficient evidence on sweat composition to permit detailed comparisons to be made or to enable any general trends to be detected save perhaps for the fact that the secretions range from the very sparse or oily to that in the horse where it is profuse and watery (Weiner & Hellman, 1960).

CONTROL OF SWEATING

Sweating appears to be under the control of the sympathetic nervous system which has been shown to be involved in the sudomotor mechanism of the cow, sheep, goat, pig, dog, donkey and horse

(Findlay & Robertshaw, 1965; Hayashi, 1968; Kimura & Aoki, 1962; Robertshaw, 1968; Iwabuchi, 1967; Cotton & Van Hasselt, 1972a, b; Robertshaw & Taylor, 1969b; Lovatt Evans & Smith, 1956). In man, sympathetic denervation abolishes the thermal response of the glands (Rawson & Hardy, 1967) and is used as a cure for excessive sweating (Rains & Capper, 1965). Evidence from the cow and dog (Ingram, McLean & Whittow, 1963; Cotton & Van Hasselt, 1972a) indicates hypothalamic involvement in the control of sweating. The sudomotor mechanism in most species appears to be an adrenergic one (Findlay & Robertshaw, 1965; Robertshaw, 1968; Robertshaw & Taylor, 1969a, b) mediated by α-receptors in the cow, sheep and goat and α- and β-receptors in the dog (Cotton & Van Hasselt, 1972a). Circulating adrenaline appears to play no part in the control of heat-induced sweating in the cow, sheep, goat and donkey but has been shown to be involved in exercise-induced sweating in the donkey (Robertshaw & Taylor, 1969b). The sweat glands of the general body surface of all mammals including man (Foster, Ginsburg & Weiner, 1970) respond to some degree to injection of adrenaline. Even those of the pig which do not respond to heat are activated by this hormone (Ingram, 1967). The glands of some mammals, e.g. the deer, cow and goat (Johnson *et al.*, 1972; Findlay & Robertshaw, 1965; Kimura & Aoki, 1962) also respond to noradrenaline but those of other species, e.g. the horse and donkey do not (Robertshaw & Taylor, 1969b). Only the sweat glands of man, the dog, Equidae, some primates and the footpads of the cat and rodents respond to acetylcholine and parasympathicomimetic agents (Aoki & Wada, 1951; Lovatt Evans & Smith, 1956; Robertshaw & Taylor, 1969b; Sakurai & Montagna, 1964). The sweat glands of the cow, sheep, goat, pig and camel do not respond to injection of these substances (Findlay & Robertshaw, 1965; Kimura & Aoki, 1962; Robertshaw, 1968; Jenkinson, 1969). The role of acetylcholine in sweating is still obscure. In contrast to the cholinergic glands of the human and primates, thermal or exercise-induced sweating in the horse and donkey is not affected by atropine suggesting that the cholinergic response in the equine is not an integral part of the normal sudomotor mechanism. In the dog Aoki & Wada (1951) found that atropine had no effect on heat-induced sweating. Recent work on beagles on the other hand (Cotton & Van Hasselt, 1972a, b) indicates that thermal sweating only occurs at high skin temperatures and that atropine blocks this activity. The comparative evidence illustrates therefore that acetylcholine is an effective stimulator in only a few species and that in the majority of animals studied although an increase in circulating adrenaline can promote sweating the sudomotor mechanism is essentially an adrenergic sympathetic one.

The sweat glands of most species studied, however, are not innervated (Jenkinson, Sengupta & Blackburn, 1966; Jenkinson & Blackburn, 1967a, b, 1968b; Bell & Montagna, 1972; Sakurai & Montagna, 1964; Jenkinson & Maloiy, 1969; Rollinson, Injidi & Jenkinson, 1972). Only the sweat glands of man, some primates, the horse and the footpads of the dog, cat, laboratory rodents and rabbit have a nerve supply closely associated with them (Rothman, 1955; Sakurai & Montagna, 1964; Machida & Giacometti, 1967; Jenkinson & Blackburn, 1968a; Jenkinson, 1970; Bell & Montagna, 1972). In the majority of species, therefore, there must be a non-neural peripheral component in the sudomotor control mechanism. Attempts have been made to test the hypothesis that the peripheral link in the cow is a humeral one involving adrenaline. The natural fluorescence of the sweat glands, however, prevented the detection of catecholamines on them (Thompson, unpublished data) and the only catecholamine stores within the skin without nerve fibres were in cells which subsequently proved to be mast cells (Jenkinson *et al.*, 1970). Mabon & Jenkinson (1971) demonstrated that catecholamine metabolites, in particular 3-methoxy-4-hydroxy mandelic acid (VMA) occur in cattle sweat. Their study, however, provided no evidence for the peripheral action of catecholamines in sweating since the amount of VMA in the sweat was greatly increased when the blood level of this substance was raised. At present therefore one can only speculate on the nature of the peripheral mechanism.

In cattle cutaneous blood flow is of importance to sweat gland activity (Ingram *et al.*, 1963) and there is in cattle skin a large arteriole situated at the level of the hair follicle bulb which unlike other cutaneous arterioles does not appear to be accompanied by a venule (Jenkinson, 1965). The most likely theory at present therefore is that the sympathetic nerves involved in sudomotor control, terminate on this blood vessel and that on stimulation they cause either a change in permeability of the arterial wall, an elevated hydrostatic pressure (Alvarez, Hahn & Johnson, 1970) or release of a transmitter from the vessel or its environs, thereby inducing sweating. From the comparative viewpoint it is interesting to note that the species with a network of nerve fibres surrounding their sweat glands, e.g. man and the equines, are those which also have a profuse capillary blood supply in close proximity to them. Sweat gland activity in man and the equine is also influenced by cutaneous blood flow (Collins, Sargent & Weiner, 1959; Kennard, 1963; Bannerjee, MacIntyre & Bullard, 1968; Bullard *et al.*, 1970) and it is intriguing to speculate on the possibility that most if not all the nerves around the sweat glands in such animals are supplying the capillaries. In such an event the mechanism of control could be basically the same in all species, the greater efficiency for profuse sweating in man and the horse resulting from the closer proximity of the vascular supply to the glands and hence a closer peripheral link in the sudomotor control mechanism as well as better access to nutrients.

D. McEwan Jenkinson

Department of Physiology
The Hannah Research Institute
Ayr, Scotland

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Correspondence

PEMPHIGUS AND MALIGNANCY

SIR, Since I reported a patient with malignant lymphoid neoplasm who later died of pemphigus vulgaris (Saikia, 1972), two other papers have been published reporting the association of pemphigus with malignant conditions. One of these papers (Saikia & MacConnell, 1972) reported three cases of Senear–Usher syndrome with internal carcinomata. The other paper (Chang *et al.*, 1972), describes a case of pemphigus vulgaris associated with epidermoid carcinoma and is of considerable importance.

This patient, a 45-year-old Formosan male, with an ulcer of 3 years' duration on his right leg, developed a generalized bullous eruption. Clinically and histopathologically his eruption was consistent with pemphigus vulgaris. Indirect immunofluorescence tests confirmed the diagnosis and the initial serum antiepithelial antibody titre was 1 : 640. The eruption responded well to 40 mg systemic prednisolone daily, but on repeated attempts to reduce the dose of systemic corticosteroids, the skin lesions reappeared when the dose of prednisolone was reduced to 20 mg daily. During this time the pemphigus antibody titre remained at its initial level. At this point it was found histologically that the ulcer on the patient's right leg showed evidence of epidermoid carcinoma, and in consequence an above-knee amputation of the affected leg was performed. Soon after amputation the anti-epithelial antibody titre was reported as negative and the patient's skin lesions gradually disappeared; systemic corticosteroids were tapered off in 2 weeks after operation without any fresh activity of the skin disease. Four months after removal of his leg, the patient was completely free from any skin disease.

This case report gives further evidence of the interrelationship of tumour tissue with pemphigus and the anti-epithelial antibodies. Although there were no attempts to extract the anti-epithelial autoantibodies—as carried out in the case reported by myself (Saikia, 1972)—the clinical history of the case strongly suggests the aetiological involvement of the tumour with the development of pemphigus antibodies and the disease in this patient. Such an interrelationship probably also existed in the three cases with Senear–Usher syndrome reported earlier.

I am informed that Dr I. B. Sneddon has under his care a patient with a tumour of the maxillary antrum, who recently developed a generalized bullous eruption with a positive Nikolsky sign. Unfortunately, this patient's general condition has not allowed any further investigations at the present time to confirm the clinical diagnosis of pemphigus.

These recent case reports, though few, suggest that pemphigus may appear as a skin marker of internal malignancy. I am not inclined to believe that every patient with pemphigus has an associated malignant condition, but I feel strongly that patients with pemphigus should be investigated as far as is practicable to exclude this possibility. This is particularly so in those cases in which the response to systemic corticosteroid therapy is unsatisfactory in controlling the pemphigus.

The production of pemphigus antibodies by a tumour is well known, as in the association of pemphigus with myasthenia gravis and thymoma (Kough & Barnes, 1964; Beutner *et al.*, 1968; Peck *et al.*, 1968; Whittingham & MacKay, 1971; Vetters *et al.*, 1973). In the light of current immunological concepts, it is not surprising that malignant lymphoid tumours may produce antibodies to skeletal muscle and epithelial tissues. Thus there is now increasing evidence of an association between epithelial malignancy and the production of antibodies to epithelial tissue. The report of the case from Formosa strengthens this view and it is interesting to speculate what changes occur in a malignant squamous cell to induce the formation of antibodies against its parent cell type.

Department of Dermatology
University of Glasgow, and
Western Infirmary
Glasgow

N.K.SAIKIA

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ATROPINE AND SWEATING

Sir, A number of reports are to be found in the literature concerning the so-called 'cholinergic' actions of atropine (Averill & Lamb, 1959; Hazard *et al.*, 1959; Ashford, Penn & Ross, 1962). These effects appear to be very dependent on the relative concentrations of atropine and acetylcholine employed. We have recently observed that dose response curves of sweating from acetylcholine injections in the dog are inhibited at high acetylcholine concentrations (Cotton & van Hasselt, 1972). We reasoned that if these dose response curves were shifted to the right by a competitive inhibitor, such as atropine, then by reducing the effects of high concentrations of acetylcholine, atropine would have an apparent cholinergic effect. Similarly at lower acetylcholine concentrations atropine would show its normal inhibiting effect. It also seemed probable that atropine would show intrinsic activity at very low doses thus making it possible to construct a dose response curve for atropine at low doses which would be comparable to

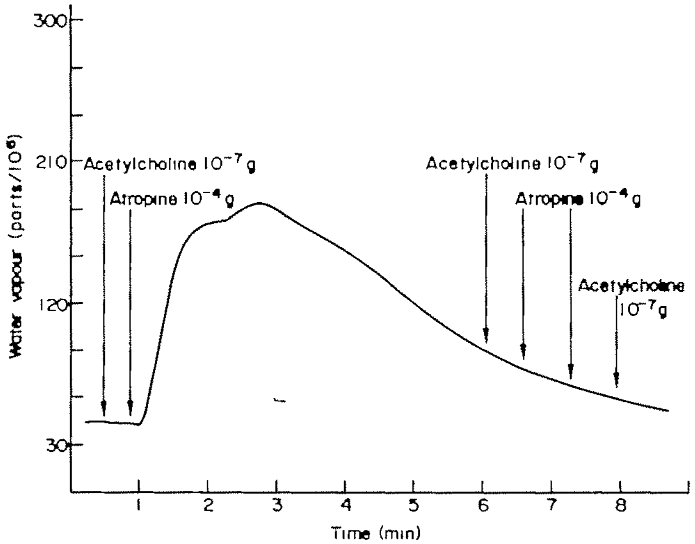

FIGURE 1. Reversal of acetylcholine high dose inhibition of sweating by atropine. Units of sweating (parts/10^6 water vapour) can be converted to g cm^{-2} h^{-1} by dividing by 40 (Spruit & Malten, 1966).

those constructed for acetylcholine and adrenaline at higher doses (Cotton & van Hasselt, 1972). In this respect it is perhaps interesting to note that a number of reports exist in the literature regarding the presence of an atropinesterase in various animal tissues (van Zutphen, 1972; Sawin & Glick, 1943).

Our experiments were carried out by injecting the substances, dissolved in 0.1 ml of physiological saline, intracutaneously into the shaved flank skin of five anaesthetized (halothane; N_2O; O_2) Beagles and recording the sweat responses using a Meeco electrolyte water vapour analyser (Spruit & Malten, 1966). The output of the Meeco was continuously recorded on a Servoger flat bed recorder. The sweat outputs were calculated as maximum sweat rates from the peak heights of the sweat response. Two types of experiments were carried out. In the first type a high, inhibitory, dose of acetylcholine was injected and after about 30 s this was followed by an injection of atropine. In control experiments the high doses of acetylcholine produced no sweat and atropine without acetylcholine also produced no response. The results of a typical experiment are shown in Fig. 1.

In the second type of experiment, 0.1 ml of various concentrations of atropine in physiological saline was injected intracutaneously and the sweat response recorded. The actual quantities of sweat produced varied from animal to animal but the optimal dose was always around 10^{-17} g atropine. A dose response from one animal is shown in Fig. 2.

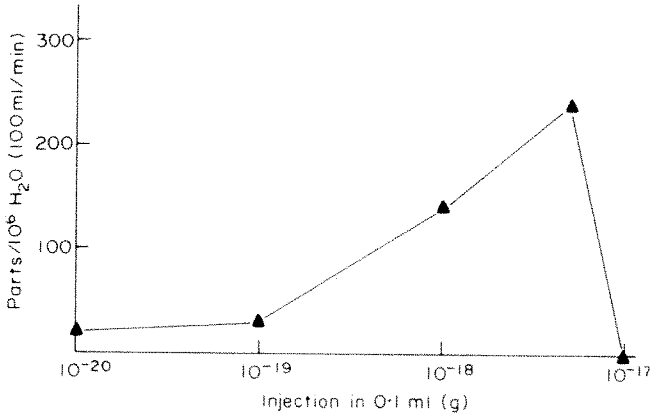

FIGURE 2. Dose response curve of sweating to atropine injections.

From these results it can be seen that atropine has intrinsic acetylcholine-like activity at low concentrations and that it acts competitively with high concentrations of acetylcholine. It is also well known that atropine is a competitive inhibitor of acetylcholine-induced and thermal-induced sweating in the human and we have previously shown that this is also the case in the Beagle (Cotton & van Hasselt, 1972). The extremely low concentrations of atropine that we have used here presumably account for the fact that the intrinsic activity of atropine has not previously been described in the literature on sweating, although it is mentioned occasionally in the pharmacological literature. Since the doses of atropine were so low as to suggest artifacts we carried out a number of control experiments with physiological saline and with various concentrations of histamine. These experiments were consistently negative. The practical significance of these results is to underline the neccessity for caution in interpreting negative results in the pharmacology of sweating, since these may arise from inhibition due to a high dosage. It would seem useful to check such negative results by a subsequent atropine injection.

Unfortunately our results in general serve to further confuse the already complex situation of the pharmacology of the sweat response.

Department of General Neurophysiology
Department of Dermatology
University of Nijmegen
The Netherlands

P. van Hasselt
D.W.K. Cotton

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Book Reviews

Keratins. Their Composition, Structure and Biosynthesis. R.D.B.Fraser, T.P.MacRae & G.E.Rogers (1972) Springfield: Thomas. Pp. 304; 105 illustrations. Price: £6.00.

This work is written by a team of experts in the field of keratin chemistry. It contains up-to-date information on the chemistry and aminoacid composition of the various types of keratins including hair, wool and feathers. The modern work on high and low sulphur fractions is adequately explained, as are the newer aspects of high glycine and tyrosine proteins in keratins. The histology of the different keratins is well covered with a number of electron micrographs of good quality which include some taken with the scanning microscope. The chapter on molecular structure of keratins gives details of X-ray diffraction patterns and a full account of the helical nature of the keratin molecule. There is a similar chapter devoted to avian and reptilian keratins, and this together with a chapter on the physical properties of keratins occupy the first half of the book.

The second part is concerned with dynamic aspects of keratinization, under the title of biogenesis of keratins. This section is well illustrated with electron micrographs of high quality, but is less comprehensively covered than the previous part. Included are sections on epidermal chalones, epidermal growth factor, vitamin A, and RNA synthesis. The mechanism of epidermal keratinization is accepted as being due to the synthesis of a histidine rich protein at a high level in the epidermis; other possible mechanisms are not considered. The evidence for high level protein synthesis in keratinizing epidermis has been derived almost exclusively from the new born rat in which the granular layer is unusually thick and extends almost to the basal layer. The reference to vitamin A is devoted to the earlier work on the mucous metaplasia of chick embryo skin, which is not a characteristic effect of this vitamin in any adult mammalian skin.

In general terms, therefore, this book is excellent on the chemical and physical nature of keratins but weaker on the dynamic aspects of the formation of keratinized structures. While it may not be of immediate interest to the clinical dermatologist, it is essential to anyone wishing to study keratin, and should also be read by all experimental dermatologists.

A.Jarrett

Steroids in Human Skin. M.Julesz, I.Faredin and I.Toth (1971) Budapest: Akadémiai Kiadó. Pp. 221, 33 illustrations. Price: £3.50

Only in the last two or three decades has it become appreciated that skin is much more than a protective cover which also plays a part in temperature control. It is well known that both the activity of the sebaceous glands and the hair structure are profoundly affected by hormones. But not all adolescents suffer from acne because of a crescendo in their androgens, and perhaps one-half of all cases of female hirsutism can be categorized as idiopathic. The skin is itself a site of intense metabolic activity. Could it be that individuals differ greatly in the way their skins utilise and react to hormones?

This work is not only an historical account of the investigations on the distribution and the metabolism of steroids in human skin, carried out for well over a decade by the Szeged group, but a valuable source for other workers of background information and methods. Starting with a clear demonstration of Zimmermann chromogens in human skin, the team carried out qualitative estimations of the amounts of cholesterol and cholesteryl esters in different regions of male and female skin, and also in hair. From axillary hair they were subsequently able to identify a range of 17-ketosteroids in the form of their water soluble sulphates. Pubic hair contained less, scalp hair none. It was established that the axillary sweat of males and females alike contained both DHA and androsterone sulphates.

These findings in turn led to studies on the metabolism of steroids by human skin *in vitro*. Male and female pubic skin was found capable of transforming 4-^{14}C dehydroepiandrosterone into a range of metabolites including 7α-hydroxy-dihydroepiandrosterone, 7-ketodehydroepiandrosterone, 7β-hydroxydehydroepiandrosterone, androst-5-ene-3β, 17β-diol, testosterone androst-4-ene-3,17-dione, 5α-androstane-3,17-dione and androsterone. Though studies with an agonadal male (karyotype: XY) revealed some additional metabolites, no enzyme deficiencies were noted. Nor, starting with labelled androstenedione as a substrate, could any qualitative differences be detected between skin from normal females and patients with hirsutism of ovarian or adrenocortical origin.

Some of the experiments described were carried out in the Royal Infirmary at Glasgow and the elegance of the English translation owes much, no doubt, to Dr F.Sharp. The book is logically organized, and contains an appendix of over 40 pages giving practical details of the methods used, a reference list and both author and subject indices. It is beautifully produced, with colour plates, by Akadémiai Kiadó, Budapest.

Dr Miklos Julesz, MD, DSc, Corresponding Member of the Hungarian Academy of Sciences and Professor of Medicine at the University Medical School of Szeged, died at the age of 68 on 18 October 1972. This field of work in which he was among the pioneers will be continued by his colleagues and others. The book remains as one memorial to a man, physician and scientist who was in the best tradition of Hungary, part of the heartland of European endocrinology.

John Ebling

Dermatologie und Venerologie für Artze und Studenten. G.K.Steigleder (1972) Stüttgart: Thieme. Pp. 243, black and white illustrations, 19 tables. Price: £2.70.

Discussions about the content of the medical curriculum are endless but are they really necessary? Books and journals in every language and of every format are getting better and better, multiplying faster than homo sapiens himself, and more than adequately plug gaps left by the teachers. Steigleder's new pocket-size paperback encompasses nearly the whole of dermatology and even includes (still under the heading of diseases of the skin) aspects of proctology, and that apparent firm favourite of German dermatology, andrology. The approach is clinical, illustrations are fair to middling, and histological findings are mentioned but not illustrated. There is, for balance, perhaps not enough basic science (here the paperback by Jarrett and Spearman seemed better) but most rarities (in contrast to English books of roughly similar intent) find mention (including, for example the 'progeroides Syndrome Moynahan').

Steigleder interpolates useful short summaries into his text. He has done a good, up-dated, job. The book will be justifiably popular in the German-speaking world.

MICHAEL FEIWEL

Herpes Simplex, Varicella and Zoster. Clinical Manifestations and Treatment. B.E.JUEL-JENSEN AND F.O.MACCALLUM (1972) London: Heinemann. Pp. 187, illustrated, tables. Price: £3.50.

The authors must be congratulated on this excellent monograph that fills an obvious present need. The basic virology of the herpes virus group has been covered recently in another monograph, and in this volume the authors have rightly concentrated more on the clinical aspects of infections with *Herpesvirus hominis* and *Herpesvirus varicellae*, and have demonstrated admirably the protean clinical manifestations of infections with these viruses.

The number of patients with incompetent immune responses due to disease, medical treatment, or both, has greatly increased in recent years, and it is not surprising that the incidence of infection with these latent and often opportunistic agents has become more of a problem. The book also deals very adequately with some basic virology and the laboratory diagnosis of herpes simplex and varicella/zoster infections. Of particular value is the final section that deals with the treatment of these conditions. Infections with these viruses are among the very few that can now be treated with anti-viral drugs, and the authors themselves have contributed significantly in the use of high concentrations of idoxuridine in dimethyl sulphoxide topically in the treatment of herpes zoster, and in the use of cytarabine for the treatment of generalized infections. On balance, the authors confirm the value of idoxuridine in the treatment of localized and systemic herpes simplex, and suggest that it may also have a place in the treatment of herpes zoster. The use of cytarabine is of great interest in the treatment of these infections, but further data will be necessary to assess its real value.

This well-written, accurate, succinct, timely monograph will no doubt be very useful to general practitioners, paediatricians, ophthalmologists, dermatologists, microbiologists, and perhaps other disciplines all over the world.

TREVOR ROBINSON

Epidermal Wound Healing. H.I.MAIBACH and D.T.ROVEE (Eds) (1972) Chicago: Year Book Medical Publishers Inc.; London: Lloyd-Luke (Medical Books) Ltd. Pp. 372+xii, illustrated. Price: £11.70.

The attempt at restoration of normal architecture after trauma is a fundamental property of almost all mammalian tissues. The healing of cutaneous wounds has been especially intensively studied both because of their practical importance to surgery and because easy access to the skin has led to the development of good experimental models. Indeed, the subject has become so replete with original papers, reviews and the reports of meetings, symposia and colloquia that is seems regrettably to have become necessary to divide off epidermal wound healing as a separately considered facet on its own. This book is a report of a conference on the epidermal aspects of wound healing that took place at Pebble Beach, California in autumn 1971. All concerned with its publication are to be commended for producing an interesting and readable account in so short a period. Of particular interest are chapters that deal with the effects of various dressings on the wound healing process, including those written by L. Fisher and H. Maibach, by G. Winter, and by D. T. Rovee and his co-workers. It appears from all these investigations that epidermal migration is increased beneath occlusive dressings. In addition, in the chapter by Rovee and his colleagues evidence is provided that there is considerably more tissue damage following air exposure than following occlusive dressing and this damage probably predisposes to scarring. Another chapter, from the same group at the Department of Skin Research at Johnson and Johnson in New Jersey, describes the effects of climate on wound healing and concludes that a high ambient humidity promotes epithelial healing. Active epidermal cell movement is discussed in several chapters; this activity, independent of keratinocyte proliferation, is increasingly recognized as an important component of the epidermal response to trauma. The ultrastructural characteristics of the migrating cell are well described by Martinez, who has used the gingival mucosa of the rat as a convenient experimental model, and by Krawczyk, who has studied the migrating epithelium in blisters. There are many other interesting contributions that merit attention including that by Billingham, and one that particularly fascinated this reviewer, on antler regeneration in deer by Goss (if only we could regrow our limbs as they regrow their antlers!). Some readers might find it irritating that related chapters are widely separated but in general this book is well worthy of attention from all those who deal with the injured and diseased skin.

RONALD MARKS

British Journal of Dermatology (1973) 88, 414.

Quarterly Review

CONTACT DERMATITIS XXII

Dermatitis from lanolin

Patch testing as a method of allergic investigation has two main implications; first it has to reveal the cause of a current or recently cured dermatitis, after which relapses of the disease can be avoided by the knowledge of the established allergens. This second prophylactic purpose is of specific importance if there is a danger that the doctor may himself apply the incriminated allergen to the skin of the patient in a medicament, thus offending against the guiding principle of the medical profession: *nil nocere*. This possibility is not at all unlikely, for 30–40% of all cases of contact dermatitis are caused, or at least aggravated by medicaments (Bandmann, 1966; Bandmann, Colman & Cronin, 1972). The effect of applied medicaments is due not only to the incorporated drugs, such as antibiotics, corticosteroids, tars and so on; the ointment base is also of essential importance, as the sensitization can be elicited not only by the main ingredients but also by the vehicle.

Since its introduction by Liebreich & Unna (Peter, Schröpl & Evanzeva, 1969) at the turn of the century lanolin has been an important ointment base, alone or in combination with other substances. It can be obtained by extracting the washing water of fleece sheared from the sheep (Cronin, 1966). Lanolin is, therefore, a chemically variable mixture of woolfats or woolwaxes, and is not of constant composition. Besides its use in medical and cosmetic preparations, as in ointments, creams, soaps, shaving creams, shampoos, it is utilized for the prevention of corrosion of metals, in the production of leather, as an ingredient of furniture polishes and shoe creams, and last but not least in printing inks and in the manufacture of some brands of paper (Cronin, 1966). There is general agreement today that the aliphatic alcohols isolated by hydrolysis with alkali are to blame for its allergic properties (Cronin, 1966; Peter *et al.*, 1969). In this connexion it is interesting that the first report of a skin reaction concerned Nivea cream which contains Eucerin, consisting of 6% wool alcohols and 94% petrolatum (Marcus, 1922). Peter *et al.* (1969) compiled the published papers on this subject up to 1967. In recent years Reichenberger (1965) has reported an investigation on 150 patients suffering from leg ulcer of whom fifty were positive reactors to Eucerin. Wereide (1965) detected eighteen Eucerin contact allergies in a group of 270 eczematous patients, but he added 5% salicylic acid to his testing material in order to enhance the reactions. By control tests with 5% salicylic acid in petrolatum he claimed to justify this procedure. In 1967 M. Bandmann investigating 4354 patients with established allergic contact dermatitis found 196 cases which showed positive patch tests to Eucerin, which is 4·03%. It should be mentioned that there was no concordance between reactions to Eucerin and to Adeps lanae, a finding which confirmed the results of Reichenberger (1965) and Wereide (1965). Moreover the number and, in particular, the percentage of lanolin positives is evidently lower (Peter *et al.*, 1969). Bandmann & Reichenberger (1957) explained this difference as an expression of the different grades of sensitization to the actual allergen, the concentration of which is lower in the natural lanolin than in the alcoholic fraction of Eucerin which consists mainly of wool alcohols. In recent years larger test series were always carried out with 30% wool alcohol in petrolatum (Bandmann *et al.*, 1972; Epstein, 1972; Fregert, Hjorth & Magnusson, 1969) as suggested by Hjorth & Trolle-Lassen (1963). Following this line Epstein (1972) could explain the puzzling difference in the reported incidence of lanolin allergy in Europe and the U.S.A. Again he pointed out that plain lanolin often produces false negative patch tests, but he also claimed that 30% wool alcohol in petrolatum sometimes causes false positive reactions. Nevertheless, there is now wide agreement that under the precondition of a careful reading, which should be made as recommended by the ICDRG (Wilkinson *et al.*, 1970) accepting only a definite erythema as positive, 30% wool alcohols in petrolatum should be the patch test material of choice for the detection of lanolin allergy (Bandmann *et al.*, 1972; Epstein 1972; Fregert, Hjorth & Magnusson, 1969).

Excluding possible occupational contact with lanolin, two clinical diagnoses in particular involve theoretically a high risk of sensitization to lanolin, since they result in a continuing need for treatment with ointments: atopic dermatitis, and so called varicose eczema of the lower leg. Cronin *et al.* (1970) showed that the proportion of atopic patients reacting to wool alcohols was comparable to that of other eczematous patients, except those with stasis eczema. Bandmann, Breit & Leutgeb (1972) even found a lower incidence of sensitization in atopic patients. This reassuring situation changes radically if we consider patients with varicose eczema or ulcers. M. Bandmann (1967) reported that thirty-one of her group of forty-three patients allergic only to Eucerin suffered from stasis eczema. A considerable proportion of all studies on lanolin allergy have, therefore, been performed on such patients (Reichenberger, 1965). Breit (1972) showed that among 326 cases of eczema of the lower leg 13·2% were allergic to wool alcohols. Braun (1970) reported 14·6% positive tests to Eucerin in seventy-five such patients tested. The overall incidence of positive patch tests is extremely high in this group of patients too, so that it can be recommended that every patient with varicose eczema of the lower leg should be patch tested, and that the tests of course include wool alcohol.

A distinct problem of lanolin allergy is due to the fact that even after ascertaining allergy the avoidance of the allergen is very difficult, sometimes even impossible. In many countries the composition of medicaments for external use is stated very incompletely on the label. In prescribing products of the pharmaceutical industry the risk of coming across an ointment base which contains lanolin or wool alcohols is very high. On the occasion of a study of the composition of corticosteroid-containing ointments and creams the authors found that about two thirds of all medicaments for which information was available contained wool alcohols. Patch testing with wool alcohols can reveal the cause of an existing dermatitis in quite a number of cases, yet in spite of the identification of the allergen the avoidance of relapses of the disease is difficult as long as there is no legal obligation to declare all the ingredients of a medicament. We too can only join Hjorth & Wilkinson (1968) who closed their first *Quarterly Review* on contact dermatitis stating: 'unless the general practitioner knows the composition of the bases of all preparations at his disposal he cannot avoid the danger of prescribing the very substance to which the dermatologist has painstakingly shown his patient to be allergic'.

REINHARD BREIT
HANS-JÜRGEN BANDMANN

Dermatologische Klinik und
Poliklinik der Universität,
D-8 München 2, Frauenlobstrasse 9,
West Germany

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British Journal of Dermatology (1973) **88,** 417.

News and Notices

British Association of Dermatology

Change of Address of Secretary

Dr J. S. Pegum's address is now The London Hospital, Whitechapel Road, London E1 1BB.

British Medical Association

Dermatologists' Sub-Committee of the CCHMS

This new sub-committee will replace the now disbanded Dermatologists' Group. It is suggested that the regional dermatological societies should elect representatives as follows:

1. Newcastle, Leeds, Manchester, Liverpool, Sheffield	1
2. East Anglia, Birmingham, Leicester	1
3. Metropolitan area	3 (including one senior registrar)
4. Wessex, Oxford, South West England	1
5. Wales	1
6. Scotland	1
7. Ireland	1

Expense-paid meetings of this new committee will normally be held twice a year on the morning of a Section Meeting of the RSM, and the terms of reference broadly are to advise the CCHMS on any matters raised in the BMA on Skin Departments and equipment, on provision of special appliances for skin patients, on standards of ancillary services and other relevant subjects not dealt with elsewhere.

Secretaries of regional societies are requested to write to the Secretary of the BMA as soon as possible.

The British Association of Dermatology

Applications are invited for the *DOWLING TRAVELLING FELLOWSHIP* which is open to registered practitioners who are British graduates and who hold a higher medical qualification. The aim of the Fellowship is to encourage evident merit in junior physicians working in dermatology, before they become established in senior academic and professional posts.

Candidates must have a suitable training and experience in clinical dermatology, in laboratory medicine and in research methods; they will normally hold an appointment in a dermatological unit or department of a Teaching Hospital in the United Kingdom. They are required to furnish an essay on the nature of the work to be carried out and indicate at what centres the necessary facilities could be provided.

The Fellowship, which is normally awarded every third year, will be tenable for one academic year and will be of value up to £2000. There is no bar to the successful Fellow seeking other monies to supplement this award if deemed necessary.

Enquiries or applications, which should be submitted before 1 January 1974, to be made to the Hon. Secretary, Dr J. S. Pegum, The London Hospital, Whitechapel Road, London E1 1BB.

University of Cambridge Postgraduate Medical School

The fourth of the Courses held at 5 yearly intervals and entitled 'Progress in the Biological Sciences in Relation to Dermatology' will take place in Cambridge from 24 to 29 September 1973. The Course is intended primarily for clinicians of considerable experience, but anyone who is interested is welcome to attend. Details of the programme and accommodation may be obtained from the Secretary of the Medical School, J.W.Woodcock, M.A., Hills Road, Cambridge.

XV International Congress of Dermatology—Mexico 1977

The Sub-Committee on Organization of this Congress would greatly welcome suggestions or proposals, which should be sent as soon as possible to Professor Antar Padilha-Goncalves, Rua Sorocaba, 464-Grupo 308, Botafogo-ZC-02-GB, Brazil.

Italian Society of Dermatology and Syphilography

The Italian Society of Dermatology and Syphilography announces that the 52nd National Congress of the Society will be held at Naples and Castellamare di Stabia from 10 to 13 May 1973.

The themes of the program will be: cutaneous drug eruptions; light eruptions; immunofluorescence in dermatology (round table).

The 53rd Congress of the Society will be held at Rome during the last week of May 1974 and the themes will be: reticuloses; autoimmune cutaneous diseases; gonococcal infection (round table).

German Dermatological Society

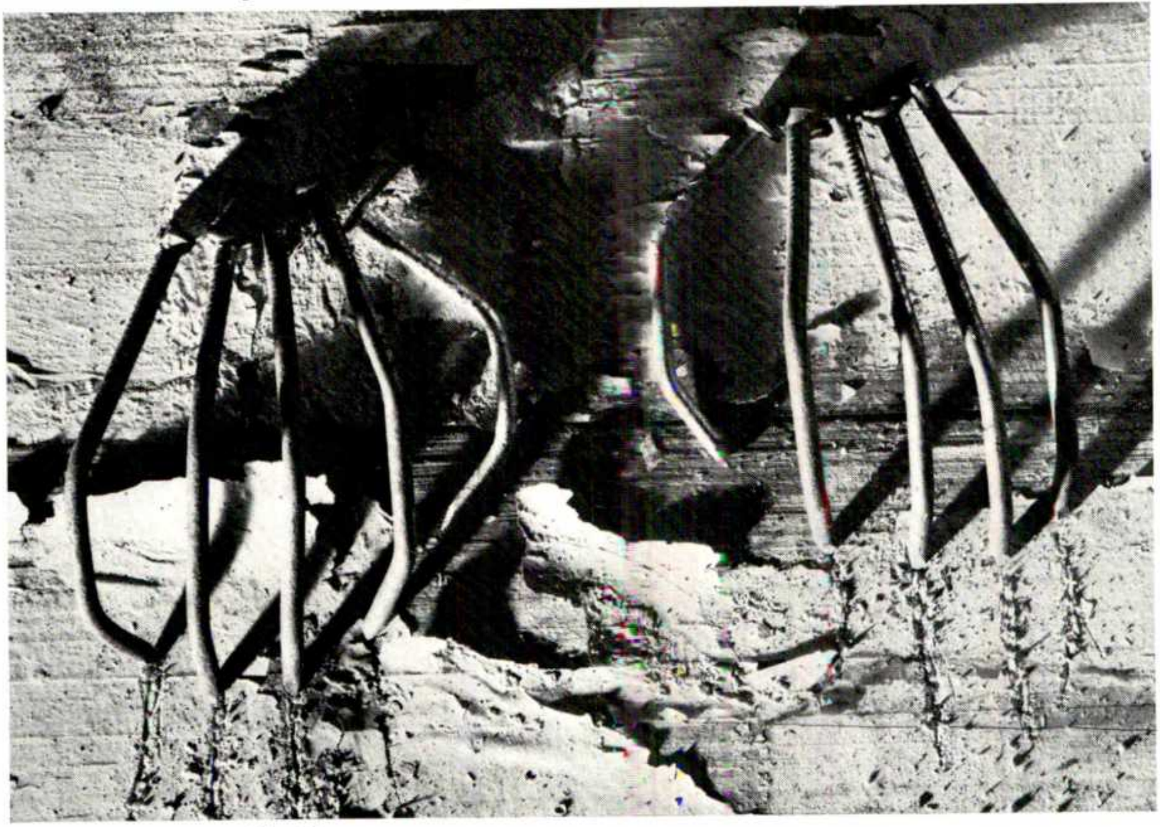
The 30th meeting of the German Dermatological Society will be held in Graz from 10 to 14 September 1974. The main topics are: progress of therapy, immunodermatology, infectious exanthemata, especially virus infections. Symposia will deal with: electron microscopy, infections of the genital area, reticuloses, and dermatological teaching.

APPOINTMENT

Western Infirmary and Southern General Hospital, Glasgow

Dr Rona M. MacKie has been appointed Consultant Dermatologist to the Western Infirmary, Southern General Hospital and Royal Hospital for Sick Children, Glasgow. Dr MacKie qualified M.B., Ch.B. (Glasgow) in 1963, was awarded an M.D. with Commendation for her thesis on 'Pre-operative Assessment of Pigmented Lesions' in 1970, and proceeded to the M.R.C.P. (U.K.) in 1971. Dr MacKie commenced her dermatological career in the Western Infirmary, where she was Registrar, and then became a Lecturer in Dermatology, University of Glasgow. She continues her research interest in pigmented skin tumours with special reference to delayed hypersensitivity in malignant melanomata.

Instead of just scratching the surface of the irritation problem



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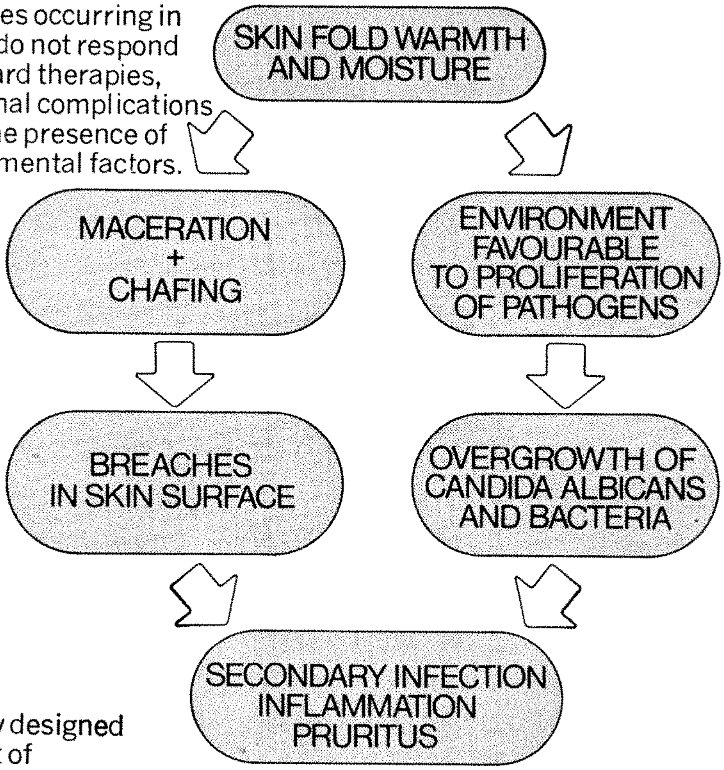
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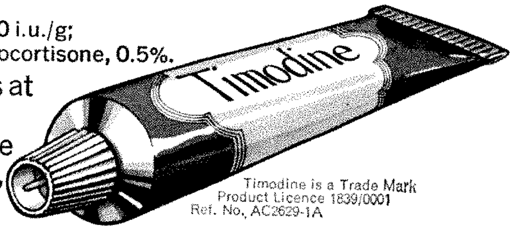

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microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	percent	%
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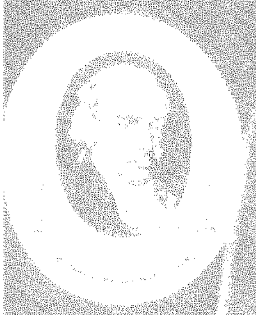

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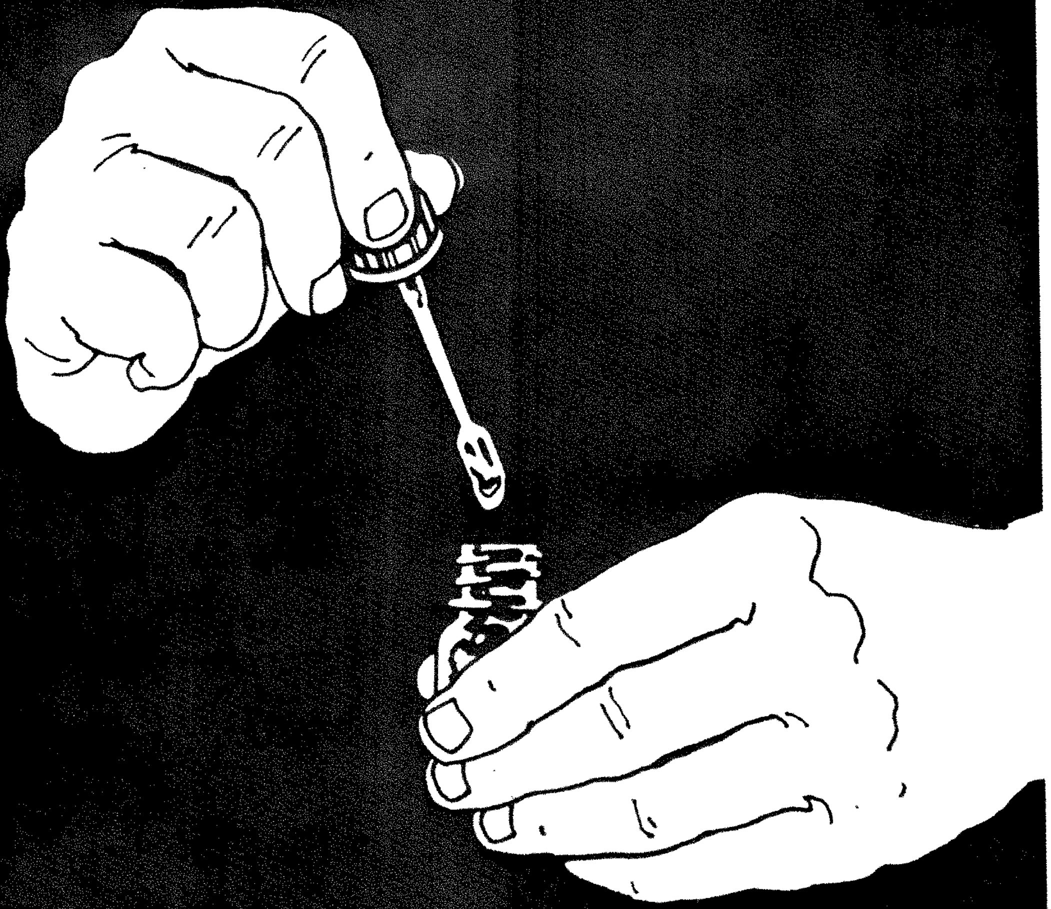

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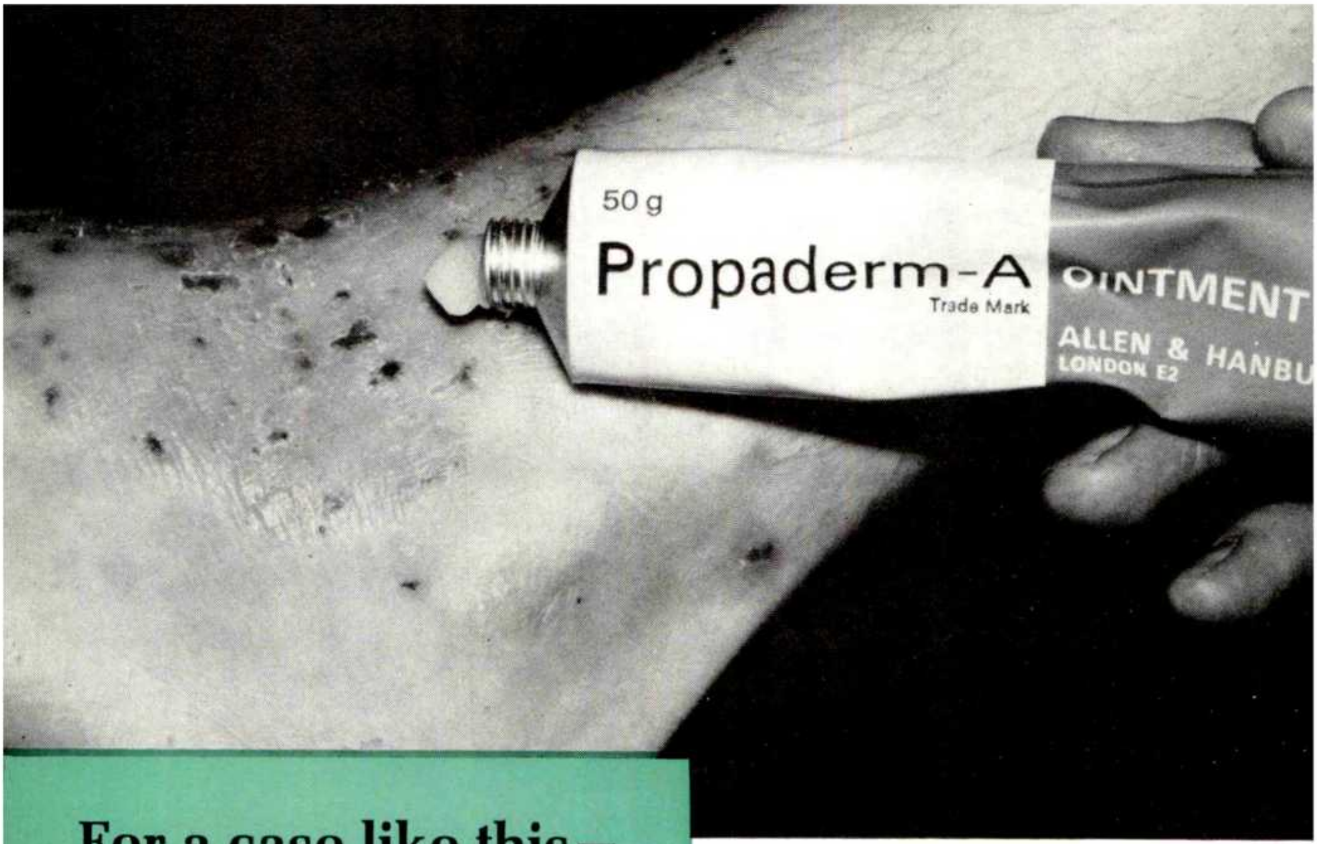

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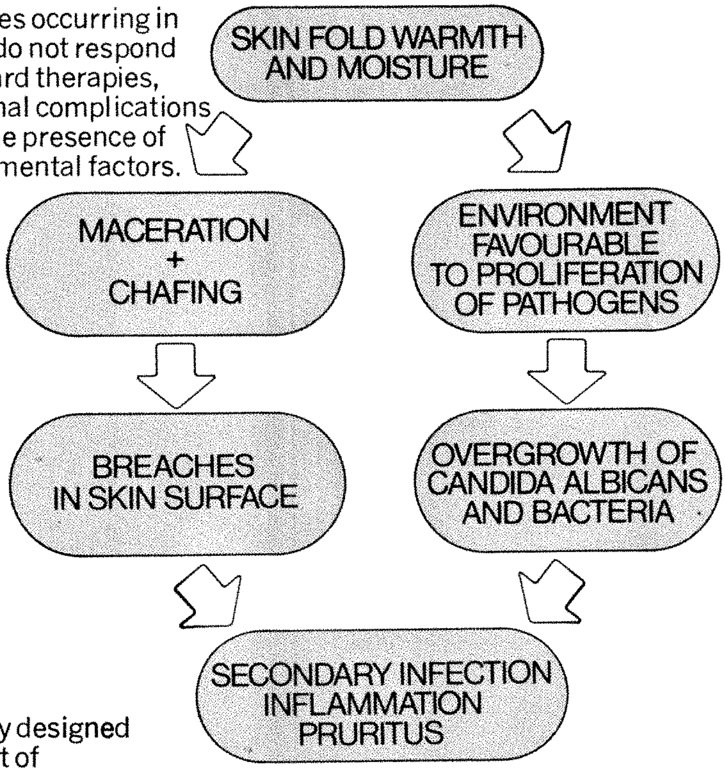
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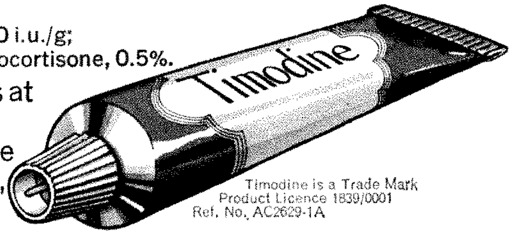

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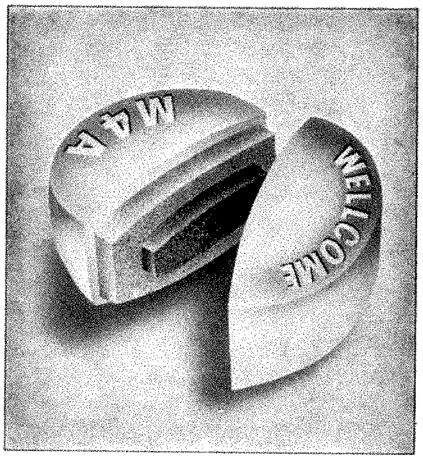

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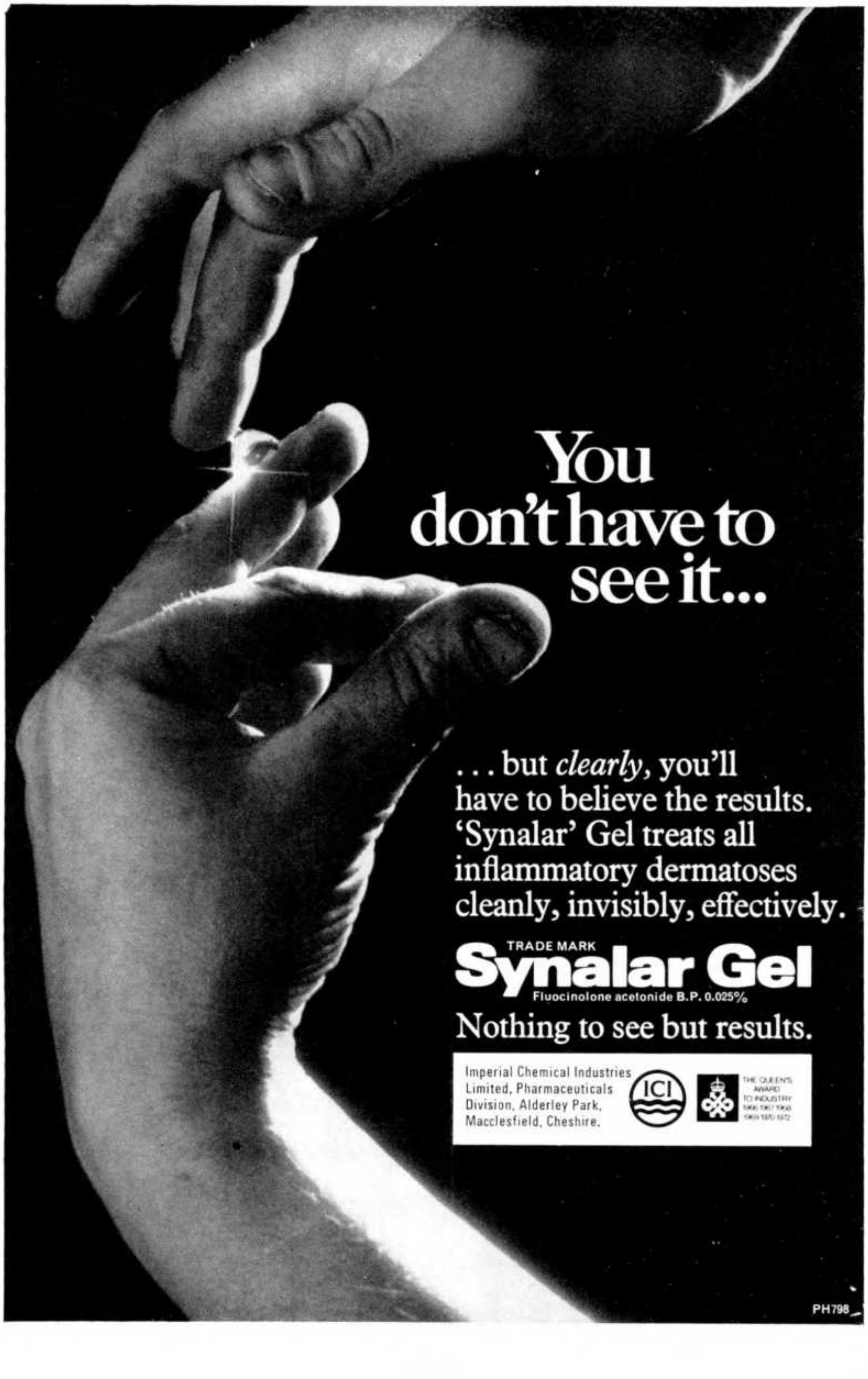

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Clinical and Laboratory Investigations

Can mycosis fungoides begin in the epidermis? A hypothesis

ELIZABETH A.RYAN, K.V.SANDERSON, P.BARTÁK AND P.D.SAMMAN

Charing Cross Hospital, Fulham, London W.6, St George's Hospital, London S.W.1, Dermato-venerologická klinika lékařské fakulty hygienické KU, Prague, and Institute of Dermatology, St John's Hospital for Diseases of the Skin, London W.C.2.

Accepted for publication 26 October 1972

SUMMARY

On the basis of clinical, histological and electron microscopical findings, we are proposing that mycosis fungoides can begin in the epidermis. Early involvement of the basal layer of the epidermis is emphasized by the pigmentary changes which herald the onset of about half the cases. The formation of new fibrous tissue above the subepidermal elastic garland and the presence of PAS-positive globules above the epidermal basement membrane, support the view that the primary change is on the epidermal side of the elastic garland and basement membrane. The predominantly epidermal distribution of the mycosis cell in many early cases suggests that it stems from a cell normally present in the epidermis. The likeliest candidate appears to be the Langerhans cell. A derivation from this cell would explain many puzzling features of mycosis fungoides and in particular the affinity of the mycosis cell for the epidermis.

Mycosis fungoides, described and named by Alibert (1806, 1832), has provided clinicians and pathologists with problems which have not yet been satisfactorily solved. Many cases evolve from a long-standing superficial dermatosis. Red discs with a sharp margin (Fig. 1), or more diffuse patches, give a clinical picture with some features of eczema or psoriasis, but the lesions are more persistent. The earliest and most reliable clue to the outcome of the disease is the appearance of poikiloderma. This varies in degree from reticular pigmentation (poikiloderma-like eruption, Samman, 1968), to pigmentation, telangiectasia and atrophy (poikiloderma atrophicans vasculare, Lane, 1921). The development of poikiloderma gives a specific character to the initial stages of about half the cases of mycosis fungoides (Samman, 1968) and is a feature of central importance that requires explanation. Later, raised, indurated plaques appear and occasionally tumours develop.

The disease usually runs a prolonged course and persists until death of the patient either from mycosis fungoides, or from an unrelated disorder. Poikiloderma may continue for over 30 years before infiltrated lesions appear. At the other extreme there are a few patients who proceed rapidly to the tumour stage of the disease. Although mycosis cells have been observed in lymph nodes in mycosis fungoides (Lutzner, Hobbs & Horvath, 1971), post-mortem examination usually reveals no lesions in internal organs (Rappaport, 1966; Allen, 1967), a finding difficult to reconcile with the view that my-

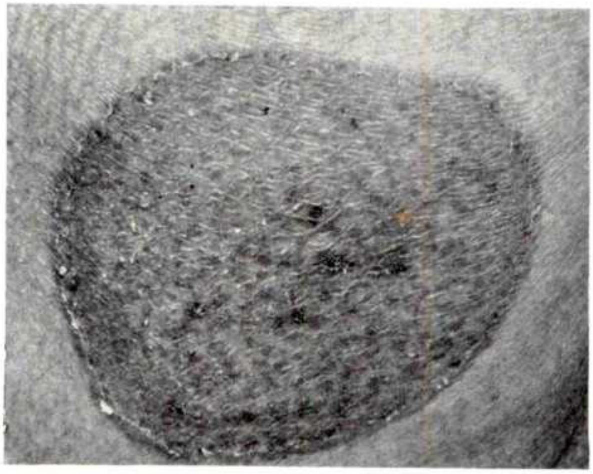

FIGURE 1. A sharply circumscribed disc of early mycosis fungoides.

cosis fungoides is simply a cutaneous expression of Hodgkin's disease, reticulum cell sarcoma, or lymphosarcoma (Symmers, 1932).

The histology may be non-specific in the early stages, but sooner or later features appear which suggest a diagnosis of mycosis fungoides. There is a lymphoreticular infiltrate in the upper dermis which is diffuse and not aggregated round blood vessels as it is in eczema. Microscopical diagnosis becomes possible with the appearance of an unusual mononuclear cell, the mycosis cell, which is distinguished by its large, dark-staining, irregular nucleus. These cells may be seen in the epidermis, dermis, or both. There have been several suggestions as to the nature and origin of the mycosis cell, based on its appearance when viewed with the light microscope (Fraser, 1925; Fraser & Schwartz, 1936; Robb-Smith, 1944; Lapière, 1964; Lever, 1967a) but recent electron microscopical investigations have not confirmed any of them (Lutzner, 1968; Brownlee & Murad, 1970; Flaxman, Zelazny & Van Scott, 1971; Lutzner *et al.*, 1971; Gomez Orbaneja *et al.*, 1972).

THE AFFINITY OF THE MYCOSIS CELL FOR THE EPIDERMIS

One of the striking features of mycosis fungoides is the affinity of the mycosis cell for the epidermis. It is generally considered that these cells invade the epidermis from the dermis, but in some cases mycosis cells appear to lie only in the epidermis and there is a relatively slight, non-specific dermal infiltrate (Fig. 2). This led us to question whether the disease begins in the epidermis, and if the mycosis cell stems from a cell normally present in the epidermis.

Biopsies from thirty-six patients with 'premycosis' or mycosis fungoides revealed a predominantly epidermal distribution of mycosis cells in eight patients (Table 1). This figure may be too low as it includes only those cases in which there was an obvious contrast between the large, deeply stained, irregular nuclei of the mycosis cells in the epidermis and the small, regular nuclei of the cells in the scanty dermal infiltrate. In many specimens it was not possible to be certain of the proportion of mycosis cells in the dermis as compared with the epidermis because of a heavy lymphoreticular dermal infiltrate. These eight patients did not show a single, distinctive, clinical feature which might suggest they were suffering from a separate disease, but they had slightly raised smooth or scaly red patches, widespread redness and scaling, hypopigmentation, or poikiloderma-like pigmentation. In other words, they were patients with early mycosis fungoides rather than severe poikiloderma, or indurated

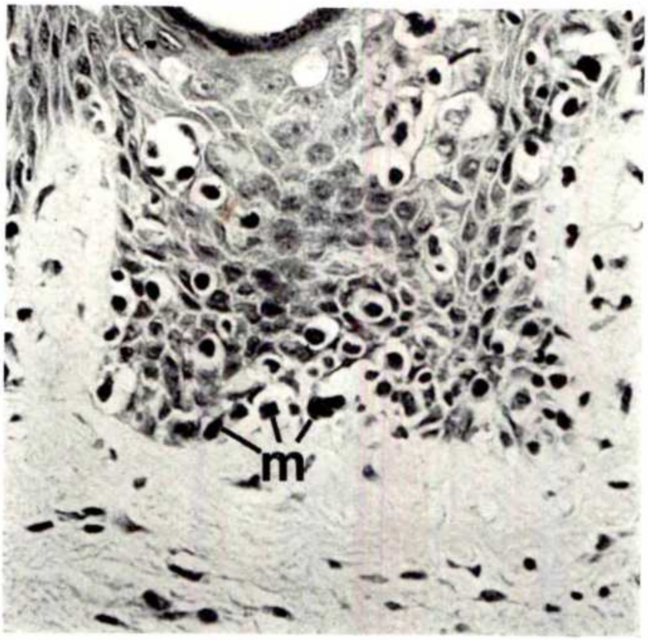

FIGURE 2. 'Premycotic' poikiloderma-like eruption. The large, darkly-stained nuclei of the mycosis cells (m) in the epidermis contrast with the smaller nuclei of the cells in the scanty dermal infiltrate (H & E, × 320).

TABLE 1. Distribution of mycosis cells in the skin in mycosis fungoides

Type of lesion	Number of biopsies examined	Predominantly epidermal distribution of mycosis cells
Slightly raised smooth or scaly red patches	12	4
Widespread redness and scaling	3	1
Hypopigmentation	1	1
Poikiloderma-like pigmentation	7	2
Severe poikiloderma (poikiloderma atrophicans vasculare)	6	0
Indurated papules, plaques, or tumours	7	0
Total	36	8

papules, plaques or tumours. In one case the mycosis cells lay in the midepidermis, but in the other seven, they were mostly in and immediately above the basal layer.

PIGMENTARY CHANGES IN EARLY MYCOSIS FUNGOIDES

Early involvement of the basal layer of the epidermis is emphasized by the pigmentary changes which herald the onset of roughly half the cases of mycosis fungoides (Samman, 1968). Occasionally there is hypopigmentation (Fig. 3). Biopsies from one such case revealed large numbers of mycosis cells in the lower epidermis, not only in the raised papules, but also in the flat, hypopigmented areas surrounding them (Fig. 4). Much more commonly there is a reticulate poikiloderma-like pigmentation (Fig. 5). Pigment accumulates in dermal macrophages. We have shown that this gives a positive result with Masson's silver nitrate technique for melanin and a negative result with the Prussian blue reaction for iron. Electron microscopy confirms that the melanin granules lie in macrophages (Ryan *et al.*, 1971).

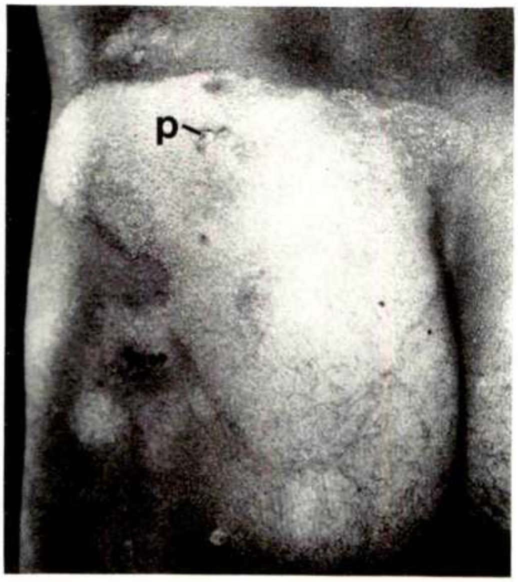

FIGURE 3. The hypopigmentation associated with infiltrated papules (p) of mycosis fungoides shows up well when photographed by ultraviolet radiation.

According to Sulzberger (1938), pigmentary incontinence occurs when there is damage to basal cells. In early mycosis fungoides, the mycosis cells above the epidermodermal junction distort the basal cells and interrupt the continuity of the basal layer. The possibility that the melanin is derived directly from degenerate melanocytes must also be considered (Frenk, 1969). With the electron microscope, we have the impression that there is a diminution in the number of melanocytes in the epidermis in mycosis fungoides.

EPIDERMAL DAMAGE IN 'PREMYCOTIC' POIKILODERMA

Further histological features focus attention on the epidermis in the pre-tumour stages of the disease. In severe poikiloderma associated with mycosis fungoides, the basal cells are vacuolated (Lever, 1967b) and the epidermis is thin. The epidermodermal junction is flattened and hyaline bodies can sometimes

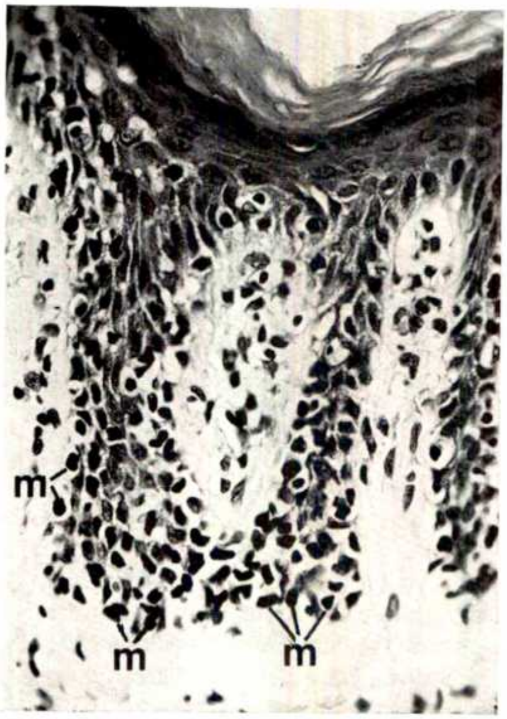

FIGURE 4. From the hypopigmented area shown in Fig. 3. Large numbers of mycosis cells (m) lie in and above the basal layer of the epidermis (H & E, × 270).

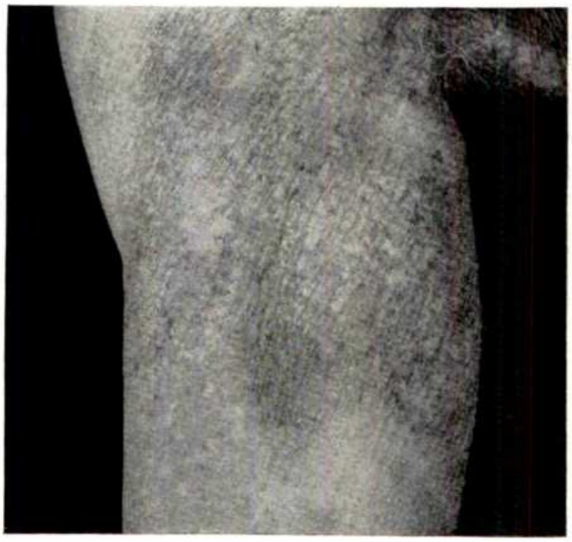

FIGURE 5. 'Premycotic' poikiloderma-like eruption showing the characteristic reticulate pigmentation.

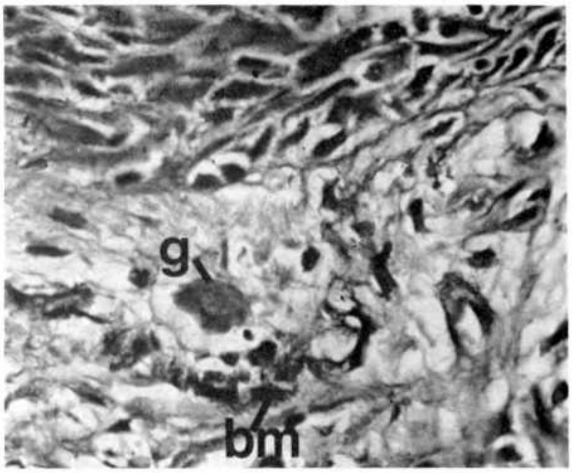

FIGURE 6. Early mycosis fungoides, from a red scaly area. A PAS-positive globule (g) lies above the epidermal basement membrane (bm) (PAS and haematoxylin, × 320).

be seen beneath it. Stoughton & Wells (1950) observed globular deposits of a PAS-positive material immediately beneath the epidermis in poikiloderma atrophicans vasculare. We have confirmed their finding in a further three cases and have also seen PAS-positive globules above the basement membrane in two cases of early mycosis fungoides (Fig. 6). Their appearance at different levels suggests that the epidermodermal junction moves from its original position, leaving the globules behind in the dermis.

SUPRAPAPILLARY FIBROSIS IN EARLY MYCOSIS FUNGOIDES

Sanderson (1965) first noted that the subepidermal garland of elastic tissue was depressed, but almost intact, in a patient with severe poikiloderma and mycosis fungoides. We have observed the same

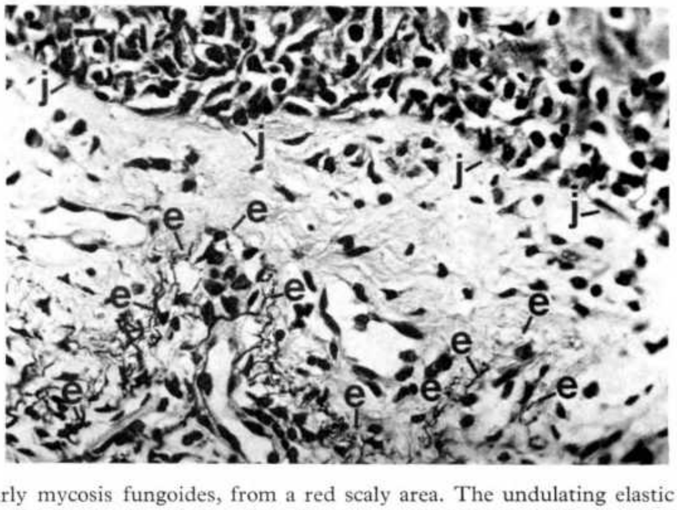

FIGURE 7. Early mycosis fungoides, from a red scaly area. The undulating elastic garland (e) is separated from the flattened epidermo-dermal junction (j) by a much wider gap than is present in normal skin (Orcein, H. & E, × 324).

change in a further four patients in the pre-tumour stage of mycosis fungoides (Fig. 7). Fine, doubly refractile fibres lie in the widened gap between the garland and the epidermis. These stain with Van Gieson's method for collagen. This sediment of new fibrous tissue is deposited above the dermal papillae and obliterates the normal papillary outline. The epidermo-dermal junction is flattened. The formation of new fibrous tissue above the elastic garland reinforces the view that the primary lesion is on the epidermal side of the garland. This fibrous tissue may simply be replacing damaged lower epidermis, or it could represent a reaction against mycosis cells above the garland. In either case, the fibrous tissue might be produced by fibroblasts or even by the epidermis itself (Hay, 1964).

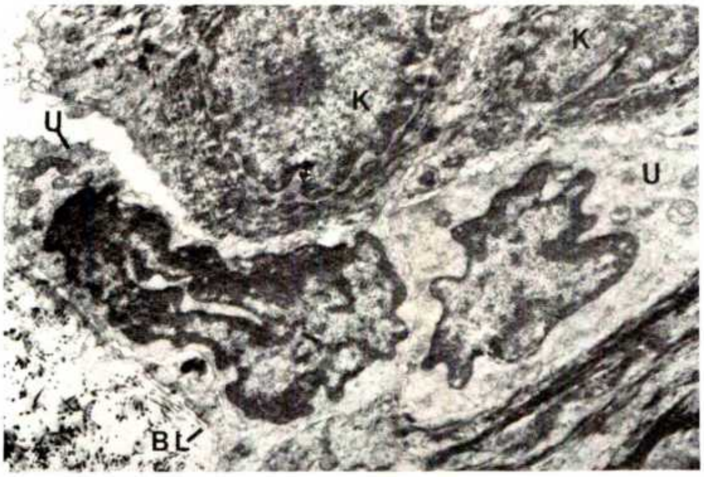

FIGURE 8. Typical Alibert type of mycosis fungoides, from a slightly raised red disc. Two non-keratinocytes (U) lie just above the basal lamina (BL). With their indented nuclei and clear cytoplasm, they have the general appearance of Langerhans cells, but lack the characteristic rod or racquet-shaped granules. K, keratinocyte (PTA stain, × 8580).

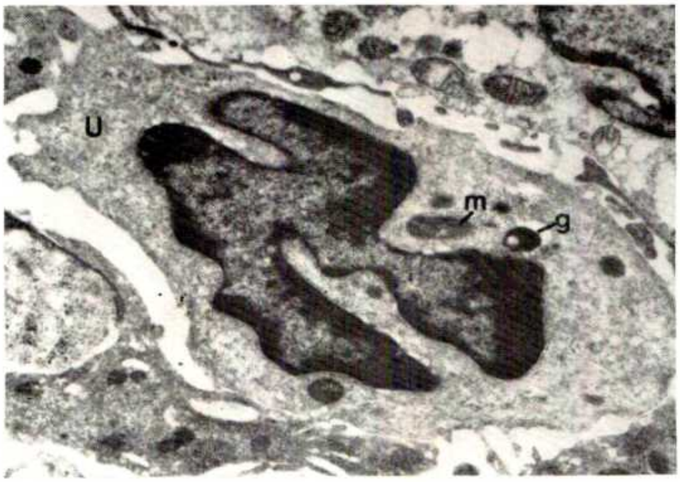

FIGURE 9. Early mycosis fungoides, from an area of poikiloderma-like pigmentation. Shows an unidentified cell (U) lying in the uppermost dermis. The heterochromatic nucleus is deeply indented and the cytoplasm contains a few mitochondria (m) and a dense granule (g) (PTA stain, × 9000).

ULTRASTRUCTURE OF UNIDENTIFIED CELLS IN EARLY MYCOSIS FUNGOIDES

We have examined four cases of early mycosis fungoides with the electron microscope. In each we found a preponderance of roughly oblong cells (Figs. 8 and 9). These had a heterochromatic nucleus with several small indentations (Fig. 8), or a few deep ones (Fig. 9). There was a relatively large amount of cytoplasm containing few mitochondria and little or no rough endoplastic reticulum. Ribosomes were arranged singly rather than in polysomes. Multivesicular bodies, fine cytoplasmic fibrils and dense granules were encountered in some cells. A few cells with more oddly shaped nuclei were also present. Some were deeply lobed; others were lobed with one straight edge, or crescent-shaped. These cells were similar in other respects to those described above and appeared to belong to the same cell line. Most of these cells in early mycosis fungoides were found in the lower layers of the epidermis and were often separated from the dermis only by the basal lamina and by a thin layer of keratinocyte cytoplasm (Fig. 8). In two cases they were also present in the uppermost dermis (Fig. 9).

DISCUSSION

The idea that foci of abnormal cells might begin to proliferate and spread within the epidermis, first provoking an inflammatory response in the dermis and only later colonizing it, is the converse of previous concepts of the origin and progression of mycosis cells. If true, however, it would explain a number of otherwise puzzling features of the disease. The prolonged inflammatory phase in which a banal infiltrate hugs the underside of the epidermis, is difficult to understand if the mycosis cells originate outside the epidermis, and to dismiss this phase as a commonplace dermatosis which somehow initiates a malignant transformation (Gall, 1955) is to ignore its characteristic and peculiar nature. The circumscribed premycotic eruptions share with the intraepidermal malignancies such as Bowen's disease and Paget's disease of the nipple, a sharp margin (Fig. 1), a red scaly surface and a prolonged and progressive course. There may even be a very close histological resemblance to Paget's disease (Brier & Steele, 1958), or pre-cancerous melanosis. The predilection of mycosis cells for the epidermis and superficial dermis and the usual absence of lesions in internal organs despite the long course of the disease contrasts with the behaviour of the lymphomas, which, when they involve the skin, often appear abruptly as an obvious dermal infiltration. Although some of these tumours, particularly the more aggressive reticulosarcomas, may invade the epidermis and ulcerate, in many of them a well-marked layer of superficial dermis is left between the infiltrating cells and the epidermis. Lymphomas commonly affect lymph nodes and other tissues. The early pigmentary changes, the PAS-positive globules above the epidermal basement membrane and the suprapapillary fibrosis could also be explained more easily if mycosis fungoides primarily affects the epidermis rather than the dermis.

Recent investigations have shown that the 'mycosis cell' is not peculiar to mycosis fungoides and that it may indeed be present in normal skin. Lutzner *et al.* (1971) have seen it with the electron microscope in lichen planus and morphologically identical cells have been detected by Flaxman *et al.* (1971) in a variety of non-lymphomatous dermatoses. Vesper, Winkelmann & Hargraves (1971), using the skin window technique, have found that the same cells migrate from normal skin as from mycosis fungoides, and on the basis of their light microscope appearance have proposed that they are benign immature reticular cells lying somewhere between a reticulum cell and a histiocyte.

The cells we observed with the electron microscope in early mycosis fungoides were not normal histiocytes, fibroblasts or endothelial cells, and although they had some morphological features in common with lymphocytes, they differed from normal lymphocytes in having indented nuclei (Ryan *et al.*, 1971). The possibility that the mycosis cell is an abnormal lymphocyte must be considered

(Barták, 1970; Flaxman *et al.*, 1971), particularly by those who hold the view that mycosis fungoides and the Sézary syndrome are the same disease (Clendenning, Brecher & Van Scott, 1964; Lutzner & Jordan, 1968). Recent studies by Crossen *et al.* (1971), point to a lymphoid origin for the Sézary cell.

Our histological studies suggested that the mycosis cell might be derived from a cell which can be found in the normal epidermis. The cells in early mycosis fungoides differed ultrastructurally from keratinocytes in having no desmosomes or tonofibrils. They were not normal melanocytes as they contained no premelanosomes or melanosomes, but it cannot be excluded that they were inactive melanocytes (Mishima, Kawasaki & Pinkus, 1972). The general appearance of these cells with their indented nuclei and clear cytoplasm would do well for Langerhans cells. The only way of identifying Langerhans cells with certainty is by seeing their characteristic rod and racquet-shaped cytoplasmic granules with the electron microscope. We did not find any of these granules in our cells, but this does not rule out Langerhans cells and might indicate that they are immature. Several cells lay close together in pairs which suggested that they had just divided. Breathnach *et al.* (1968) found only a few rod-shaped granules in the Langerhans cells of foetal mouse skin experimentally deprived of its neural crest component, and in many sections cells which otherwise resembled Langerhans cells contained no granules at all. Wolff (1967) observed no granules in Langerhans cells during the first 10 days after stripping the epidermis.

The current view is that the Langerhans cell is mesenchymal in origin (Wolff, 1967) and migrates to the epidermis, where it normally exists as a mainly suprabasal dendritic cell (Langerhans, 1868). Langerhans cells have also been found in mucous membranes near body openings (Wolff & Winkelmann, 1966; Bourlond, Wolff & Winkelmann, 1967) and in the oesophagus and bladder (Fritsch & Schellander, 1971a and b). Langerhans cells have been seen in the normal dermis (Zelickson, 1965; Kiistala & Mustakallio, 1968) and granules characteristic of Langerhans cells have been reported in lymph nodes (Jimbow, Sato & Kukita, 1969; Shamoto, Kaplan & Katoh, 1971). In histiocytosis X, Langerhans cell granules are found not only in the juxta-epidermal 'histiocytes' of Letterer Siwe disease (Cancilla, Lakey & Carnes, 1967), but also in eosinophilic granuloma of lung (Basset & Turiaf, 1965) and bone (Basset *et al.*, 1965). Langerhans cells are capable of phagocytosis (Mishima, 1966) and have enzymes in common with macrophages (Campo-Aasen & Pearce, 1966). Autoradiographic studies have shown that the Langerhans cell is capable of division (Giacometti & Montagna, 1967) and strongly suggest that this division can take place within the epidermis (Schellander & Wolff, 1967).

Most of the Langerhans cells in normal epidermis are suprabasal, but considerable numbers can occur in the basal layer, for example in vitiligo (Birbeck, Breathnach & Everall, 1961) where they appear to replace melanocytes. Although many of the mycosis cells in early mycosis fungoides lie in the basal layer, it seems unlikely that they are simply replacing melanocytes as they are present in such large numbers and may be found in the dermis as well as in the epidermis. The presence of oddly shaped nuclei suggests that the cells are altered in some way, but we cannot say whether they are benign or malignant. Others looking at the ultrastructure of lymphomas have experienced the same difficulty (Bernhard & Leplus, 1964).

In those early cases where there is a non-specific dermal infiltrate, but no mycosis cells are seen, the Langerhans cell might already have undergone the first stage in a progressive transformation without exhibiting any obvious morphological change. As the disease develops, the variety of patterns of mycosis fungoides could reflect a varied cellular response to altered Langerhans cells, perhaps analogous to that seen in Hodgkin's disease, where the abnormal histiocyte provokes different histological reactions (Lukes, Butler & Hicks, 1966). Thus, longstanding poikiloderma with marked fibrosis might be the equivalent of nodular sclerosis, whereas cases which develop tumours rapidly might be comparable with the lymphocyte depletion type of Hodgkin's disease. Between these two extremes lie the majority of cases of mycosis fungoides in which components of a lymphocytic and histiocytic reaction, fibrosis and mycosis cell hyperplasia can be found in varying proportions.

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Neurofibromatosis in pregnancy

GARDEN H. SWAPP AND ROBERT A. MAIN

Department of Obstetrics and Gynaecology
Aberdeen Maternity Hospital, and Department of Dermatology
Aberdeen Royal Infirmary

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SUMMARY

Eleven pregnant patients with neurofibromatosis have been studied. They have had twenty-four pregnancies. Ten of these patients developed hypertension during their confinements. In the eleventh patient records were inadequate for analysis. In five of the ten patients, lesions of neurofibromatosis appeared for the first time during pregnancy. In the others the lesions increased in size and number. The lesions regressed considerably after delivery. It would seem that the hypertension during these pregnancies is more than a chance association.

The cutaneous manifestations of neurofibromatosis are well recognized.

In 1882 Von Recklinghausen described the disease at length and was the first to offer the accepted theory of the pathology of neurofibromatosis.

The disease occurs one in 2500–3000 births (Crowe, Schull & Neel, 1956), and is transmitted by an autosomal dominant gene.

Skin nodules may appear during infancy or childhood but their first appearance is more frequent during puberty. Pregnancy may provide a growth stimulus (Sharpe & Young, 1937; Moritz & Snider, 1962). Brickner (1906) called fibroma molluscum gravidarum the lesions which appeared for the first time in pregnancy. He noted that they gradually disappeared after delivery.

Café-au-lait patches were not given great significance in the disease complex until the publication of Marie & Bernard, and Chauffard's papers in 1896. Crowe & Schull (1953) found that 10% of persons with no other manifestations of neurofibromatosis had one or more café-au-lait spots but spots were noted in over 90% of patients with neurofibromatosis. Whitehouse (1966) examined a group of children under 5. He considered the presence of four or more café-au-lait spots to be diagnostic in this age group. In 1964 Crowe suggested that the axillary freckling in 20% of patients presents another aid to early diagnosis.

Von Recklinghausen's disease is a multifacetted syndrome (Schönebeck & Ljungbert, 1970). Along with the cutaneous changes any somatic, cranial or autonomic nerve may be involved. General physical development may be impaired and it is a rare patient who does not show some osseous change (Crowe *et al.*, 1956). Many endocrine disturbances including acromegaly, Addison's disease, hyperparathyroidism, and gynaecomastia have been noted as well as gastro-intestinal lesions and involvement of the lower urinary tract. Oligophrenia occurs in 60% of cases (Rook, Wilkinson & Ebling, 1968; Saxena, 1970).

Vascular lesions are described as is their association with hypertension (Wallis, Deutsch & Azizi, 1970). There is a known association between neurofibromatosis and phaeochromocytoma (Healey & Mekelatos, 1958). Swapp & Main (1969) presented a preliminary report on three pregnant patients with neurofibromatosis and hypertension to the North British Dermatological Association. Since then we have studied all the known pregnant patients with neurofibromatosis in this region.

CLINICAL MATERIAL

Eleven patients were found and their records studied.

A diagnosis of neurofibromatosis was made on the presence of more than six café-au-lait spots. Axillary freckling was found in all cases. Those patients who initially had no nodules had developed them by the third trimester.

Hypertension is defined as a systolic pressure of 140 mmHg or more and/or a diastolic pressure of 90 mmHg or more on two or more occasions separated by 24 h. The blood pressure was taken on the upper arm using a mercury sphygmomanometer, the patient being recumbent and at rest.

In one patient, records of her first two pregnancies were not available, her third and fourth pregnancies were not adequately documented and her fifth pregnancy was terminated at sixteen weeks on socio-economic grounds. The skin manifestations of neurofibromatosis appeared between the fourth and fifth pregnancy.

The records of the remaining ten patients were available for each of their nineteen pregnancies.

TABLE 1. Neurofibromatosis in pregnancy—primigravida

	Systolic	Diastolic
First ante-natal visit	137 (17·4)	85·7 (14·5)
Before onset of labour	149 (10·8)	101·4 (11·4)
Post-natal clinic	140 (24·2)	83 (13·7)

Mean weight 60·2 (10·2) kg, mean height 155·5 (7·5) cm. BP in mmHg, standard deviation in brackets.

RESULTS

Blood pressure changes

Five of the ten patients in their first pregnancies had normal blood pressure readings when first seen, the other five were hypertensive. Despite attempts at treating the hypertension with hypotensive drugs or bed rest or sedation or all three, by the week before delivery all ten patients had shown a clinically significant rise in the mean systolic and diastolic pressure compared with their early pregnancy values.

At the post-natal clinic 6–8 weeks post-partum the fall in the mean diastolic pressure from its pre-labour level is again clinically significant (Table 1).

Subsequent pregnancies occurred in four of the ten patients, there being four second, three third and two fourth pregnancies. In all pregnancies the systolic and diastolic pressure before the onset of labour showed a clinically significant rise over the early pregnancy values.

Skin lesion changes

Both pigmented and nodular lesions increased in size and number during pregnancy in all patients and in seven there was considerable regression of nodular lesions following delivery. The nodular lesions if appearing for the first time were more commonly found in the scapular region and over the anterior abdominal wall; the majority were soft bluish nodules about 1 cm or less in diameter. In nine of the ten patients, biopsy of a solitary lesion confirmed the diagnosis of neurofibromatosis. In the remaining patient no biopsy was taken.

All patients were noted to have axillary freckling, though in one only one axilla was involved.

TABLE 2. Neurofibromatosis in pregnancy—second, third and fourth pregnancies

	Systolic	Diastolic
First ante-natal visit	132·2 (14·6)	82·3 (7·0)
Before onset of labour	151·7 (15·3)	103·0 (8·3)
Post-natal clinic	132·8 (13·9)	83·7 (10·5)

Mean weight 58·4 (3·9) kg, mean height 152·7 (2·6) cm. BP in mmHg, standard deviation in brackets.

Investigations

No excess of catecholamine excretion has been demonstrated in this group of patients during pregnancy.

Intravenous pyelography showed no abnormality in eight. In one the pyelogram suggested the possibility of right renal artery stenosis which has been confirmed by arteriogram. Two patients did not attend.

Slight proteinuria was noted in two first pregnancies and two second pregnancies just prior to delivery. This was not related to the degree of hypertension.

Blood urea and serum electrolyte investigations revealed no abnormality.

Oedema was not a feature in any pregnancy.

DISCUSSION

Swapp & Main (1969) presented three patients with neurofibromatosis who had developed a significant rise in blood pressure during their first pregnancy. Since then a further seven first pregnancies in patients with neurofibromatosis have been studied. In all, there was a clinically significant rise in the systolic and diastolic blood pressure. The probability of a primigravida in this region developing significant hypertension is 0·3 (derived from analysis of all case records for booked single birth confinements in the city of Aberdeen during the period of study). Thus, the likelihood of seven consecutive primigravid patients developing hypertension is less than 1 in 1000. The probability of a woman in her second, third and fourth pregnancies developing hypertension is 0·16. It seems that the development of hypertension in a pregnant woman with neurofibromatosis is more than a chance association.

Vascular changes with hypertension in neurofibromatosis are well documented though rare, and most patients with neurofibromatosis do not have hypertension (Cornell & Kirkendall, 1967). Halpern & Currarino (1965) reviewed eight cases of neurofibromatosis with renovascular hypertension and added two. Bilateral involvement of the renal arteries was noted in five patients, four of whom had coarctation of the aorta as well. Leumann *et al.*, (1970) recorded a 9½-year-old boy with hypertension and neurofibromatosis who had bilateral renal artery stenosis. Wallis *et al.*, (1970) described an 11-year-old boy with neurofibromatosis and hypertension with renal artery stenosis. Histological examination of the stenosed vessel showed a fibromuscular hyperplasia.

Bourke & Gatenby (1971) commented on fifteen cases of renal artery stenosis associated with hypertension and neurofibromatosis in the European literature and added a further case. In addition to stenosis of the main renal artery an unusual endothelial proliferation of the intima with atrophy of the media and elastic layers of the arterioles was shown.

Reubi (1944) discussed the vascular changes in neurofibromatosis as it involves the smaller arteries and arterioles. He described a pure intimal form, an intimal aneursymal form and a peri-arterial nodular form. To these Feyrter (1949) added an epithelioid and advanced intimal form.

These changes occur most commonly in the kidney, endocrine glands, heart and gastro-intestinal tract. The incidence of these changes in the vessels in neurofibromatosis is not known, but it is possible that they are common or even constant. Khalatbary (1959) noted fourty-four published cases. In only twelve of these was the blood pressure recorded and it was elevated in six.

We are uncertain why our patients developed hypertension. It could be postulated that the vascular changes of neurofibromatosis can be induced by pregnancy for the first time or become more marked along with the skin lesions. During pregnancy there is an increase in plasma volume, red cell mass and cardiac output (Paintin, 1962; Hytten & Paintin, 1963). It is unlikely that arterioles showing atrophy of the media and elastic layers would expand normally to accommodate these changes leading to a relative stenosis to the organs supplied.

We found no evidence of increased excretion of catecholamines though neurofibromatosis and adrenal medullary tissue have a common embryological origin in the neural crest.

Phaeochromocytoma occurs in association with neurofibromatosis; it is estimated that neurofibromatosis is present in approximately 5–25% of phaeochromocytomas (Healey & Mekelatos, 1958; Chapman, Kemp & Talliaferro, 1959). Pregnancy appears to be rarely associated with phaeochromocytoma but Geisler & Lloyd in 1963 referred to the thirty-seven cases reported up to that time and added their patient whose pregnancy was complicated by invasive phaeochromocytoma and, in addition, neurofibromatosis.

Obviously this group of patients requires further study. Only limited investigations could be carried out because of the associated pregnancy. Shizume & Lerner (1954) showed that melanocyte stimulating hormone levels were increased by the second month of pregnancy and continued to increase to term. It is possible that the pigmentary changes could be due to this, though there is evidence that other steroid hormone changes in pregnancy could be responsible. The increase in size of the neurofibromatous nodules remains unexplained but the regression post-partum would seem to indicate that the changes of pregnancy exert some influence.

ACKNOWLEDGMENT

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Pemphigus vulgaris and myasthenia gravis

J.M.VETTERS, N.K.SAIKIA, JANE WOOD AND J.A.SIMPSON

University departments of Dermatology and Pathology, Western Infirmary, Glasgow and the Institute for Neurological Sciences, Glasgow

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SUMMARY

This paper describes the occurrence of pemphigus vulgaris in a 46-year-old female with a thymic tumour. The myasthenia gravis went into remission within a few months of thymectomy, but the patient continued to need steroids for pemphigus vulgaris for a further 5 years. The histological appearances of the thymic tumour and skin and the serological findings are described.

Myasthenia gravis (Simpson, 1960) and skin diseases of the pemphigus/pemphigoid group (Beutner & Jordon, 1964; Beutner *et al.*, 1965) are both considered to have an autoimmune basis. It is known that individuals with one autoimmune disease are more likely to develop other autoimmune diseases (Anderson *et al.*, 1964). Although immunological and clinical studies have been performed on large numbers of patients with myasthenia gravis, the simultaneous occurrence of myasthenia gravis and autoimmune skin disease has rarely been described. The literature in English and French contains only three reports (Wolf *et al.*, 1965; Beutner *et al.*, 1968; Peck *et al.*, 1968). No details are given of Wolf's case. The subject described by Beutner *et al.* (1968) was a 45-year-old male with a thymic tumour who developed myasthenia gravis 1 year before he was diagnosed as having pemphigus erythematosus. Peck *et al.* (1968) described four cases of myasthenia gravis associated with pemphigus vulgaris; one of these was known to have a thymic tumour.

In addition to these patients with both pemphigus and myasthenia gravis, other reports have described the detection of skin antibodies in a small proportion of myasthenic patients who had no detectable skin lesions (Thivolet, Bejum & Andre, 1970; Whittingham & MacKay, 1971).

We wish to describe in detail a further case in which both myasthenia gravis and pemphigus vulgaris were present (this case was previously listed by Simpson (1970, 1971).

METHODS

(*a*) *Immunological*

The tests for epidermal antibody were performed according to the method of Beutner *et al.* (1968, 1970). Human vaginal epithelium was used as a substrate.

Tests for other autoantibodies were performed as previously described (Vetters, 1967).

(*b*) *Histological*

Tissue for microscopic examination was fixed in 10% formol saline and embedded in paraffin wax. Sections were cut 6 μm thick and stained with a variety of conventional stains.

CLINICAL DETAILS

The patient, a 46-year-old Jewish female, developed painful mouth ulceration and nodular thickening on the dorsum of her hands in February, 1966. Four months later she developed dysphagia and subsequently noted slurring of speech and ptosis. After some delay she sought medical help and was admitted to hospital in December, 1966. She was diagnosed as having a thymic tumour and myasthenia gravis and in January 1967 underwent thymectomy. Shortly after operation she developed a generalized bullous eruption which was particularly severe round the thoracotomy scar. The eruption was biopsied (see below).

Post-operatively her myasthenia gravis rapidly remitted and since July, 1967 she has not taken anticholinergic drugs. Her pemphigus vulgaris did not improve so dramatically and it was not until July 1972 that it was possible to discontinue steroids. She continues to have oral ulceration due to monilial infection.

BIOPSIES

(*a*) *Thymoma*

This measured 10 × 8 × 7·5 cm. Microscopic examination showed that the predominant cell type was the spindle shaped epithelial cell. In places, especially close to small blood vessels, the tumour cells were more ovoid in shape. Sparse numbers of lymphocytes were present. The appearances are illustrated in Fig. 1.

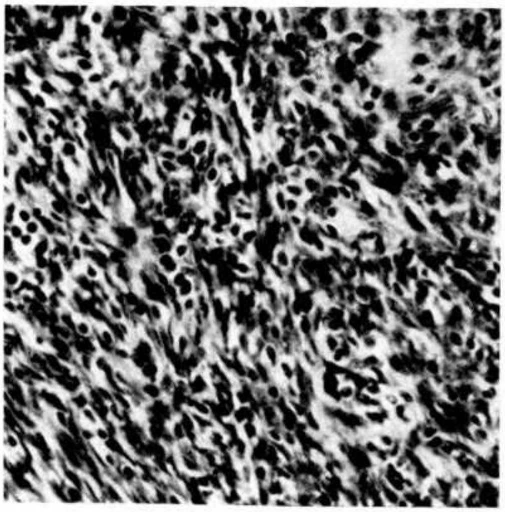

FIGURE 1. Thymic tumour. The predominant cell type is the spindle shaped epithelial cell. Relatively small numbers of lymphocytes are scattered amongst the epithelial cells. It is more common for the epithelial cells of thymic tumours to be plump when thymomas are associated with myasthenia gravis (H & E, × 250).

(*b*) *Skin biopsy*

One of the skin lesions was biopsied before thymectomy. Histological examination detected suprabasal clefts in the epidermis, dyskeratosis and giant cell formation. A mild leucocytic infiltrate was present in the underlying dermis. The appearances were found difficult to interpret and it was not until a

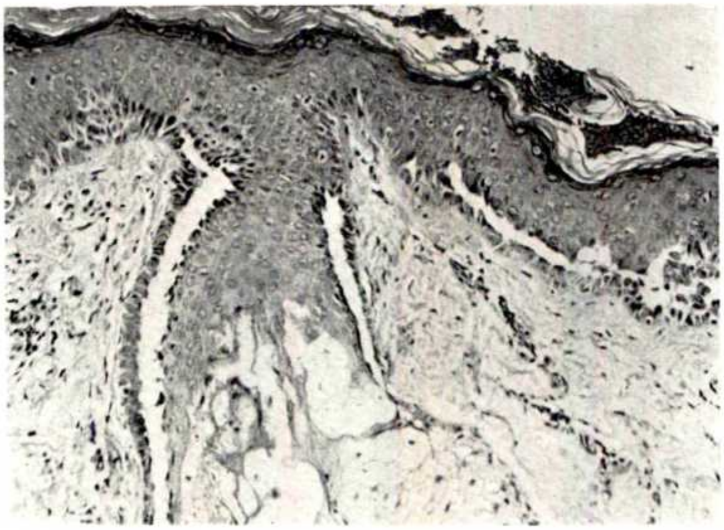

FIGURE 2. Skin biopsy. This section shows the characteristic suprabasal intra-epidermal cleft formation of pemphigus vulgaris. Notice that the clefting also affects a hair follicle (H & E, × 100).

TABLE 1. Autoantibody test results pre-operatively

Test	Result	Titre
Epidermal antibody	Positive: 'intercellular' antibody	1/50
Muscle antibody	Positive: I band antibody	1/60
Nuclear antibody	Positive	1/64
Gastric parietal cell antibody	Negative	
Thyroid microsomal antibody	Negative	
Thyroglobulin antibody	Negative	

TABLE 2. Alterations in titre of intercellular antibody

Date	Titre	Steroid dosage (mg prednisolone/day)
January 1967	1/80	15
May 1968	1/10	10
March 1969	1/40	10·8
March 1970	1/10	3·5
March 1971	1/10	2·5
March 1972	<1/10	2·5
July 1972	<1/10	Nil

second biopsy was performed, after the post-operative development of the generalized eruption, that a confident diagnosis of pemphigus vulgaris could be made (see Fig. 2).

IMMUNOLOGICAL STUDIES

The results of the pre-operative and post-operative tests are shown in Tables 1 and 2 respectively. The demonstration of intercellular antibody by immunofluorescence is illustrated in Fig. 3.

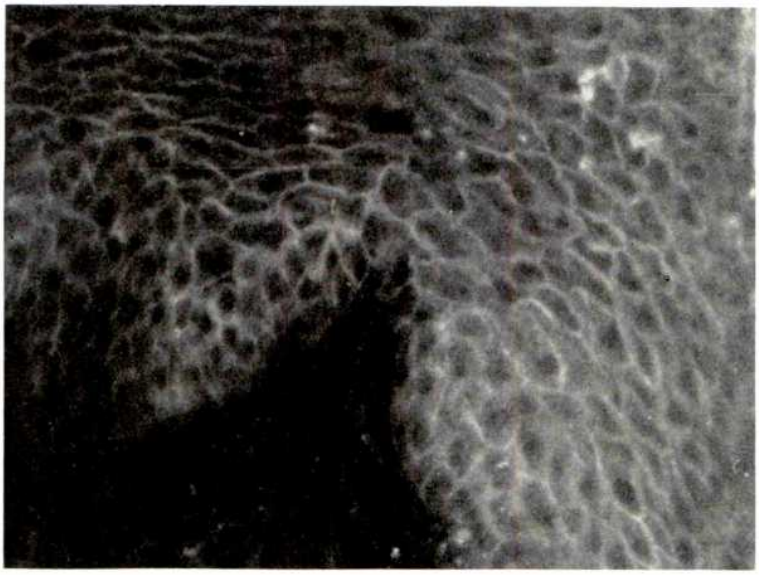

FIGURE 3. Intercellular antibody. The characteristic staining pattern caused by binding of antibody to cell membranes of the granular cell layer is illustrated (Guinea-pig lip, × 250).

DISCUSSION

It is recognized that the occurrence of one autoimmune disease increases the likelihood of developing other autoimmune diseases (Anderson *et al.*, 1964). Autoimmune disorders fall into two groups, the organ-specific, e.g. autoimmune thyroiditis, idiopathic Addison's disease and pernicious anaemia, and the non-organ-specific such as systemic lupus erythematosus, progressive systemic sclerosis and rheumatoid arthritis. A patient with an organ-specific autoimmune disorder stands an increased chance of developing another specific autoimmune disease. Similarly, subjects with non-organ-specific autoimmune diseases are more likely to develop other diseases of this group.

The position of both pemphigus vulgaris and myasthenia gravis with regard to these groups is somewhat anomalous. Pemphigus vulgaris is an organ-specific autoimmune disease, but the autoimmune disease most frequently associated with it is systemic lupus erythematosus. Myasthenia gravis appears to occupy an intermediate position between the organ-specific and the non-organ-specific groups. Patients with myasthenia gravis have an increased incidence of organ-specific autoimmune diseases, e.g. primary myxoedema and thyrotoxicosis (Feinberg, Underdahl & Eaton, 1957; Simpson, 1964) pernicious anaemia (Simpson, 1960; Howard, Silverstein & Mulder, 1965) and also of systemic lupus erythematosus (Wolf, 1966). It is therefore reasonable to expect that cases of pemphigus vulgaris and myasthenia gravis should co-exist in the one individual. What is surprising is the very small number of patients who have both disorders. As stated above, the present case is only the seventh reported in the English language. Of these, three have been associated with a thymic tumour. Unfortunately no details of the histological appearances are given in the other two reports and it is therefore not possible to determine whether any particular type of thymoma is associated with the simultaneous occurrence of myasthenia gravis and pemphigus vulgaris. It should be noted, however, that the predominantly spindle celled epithelial thymoma encountered in the present case is not the type usually found in myasthenic patients. When thymic tumours are present in myasthenic patients they usually have plump epithelial cells (Castleman, 1966). However, the thymic tumour associated with systemic lupus erythematosus and pemphigus vulgaris described by Kough & Barnes (1964) was similar to that in our case. It is therefore possible that spindle celled thymic tumours have a special relationship to pemphigus vulgaris.

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Ontogeny of pemphigus and bullous pemphigoid antigens in human skin

H.K.MULLER, RENATE KALNINS AND R.C.SUTHERLAND

Department of Pathology, Monash University Medical School,
Alfred Hospital, Melbourne, Australia

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SUMMARY

The development of pemphigus and bullous pemphigoid antigens in twenty-nine human foetuses aged 9–40 weeks has been examined by immunofluorescence. Pemphigus antigen was weakly demonstrable in the epidermal intercellular area at 9 weeks and strongly after 12 weeks. Bullous pemphigoid antigen was weak and patchy at 12 weeks and only consistently demonstrable with bright linear immunofluorescence of the basement membrane zone after 23 weeks. Morphologically detectable epidermal basement membrane is already present by 9 weeks gestation.

Patients with the bullous skin diseases pemphigus and bullous pemphigoid possess circulating autoantibodies which react respectively with antigens in the intercellular areas and basement membrane zone of mammalian stratified squamous epithelium (Beutner, Jordon & Chorzelski, 1968). Besides their diagnostic value these antibodies have been used to demonstrate antigenic loss in human (De Moragas, Winkelmann & Jordon, 1970a, b) and experimental skin cancer (Muller & Sutherland, 1971). In these studies antigenic loss, particularly of the pemphigus antigen, was correlated with impaired squamous differentiation, while areas of well differentiated tumour cells retained both antigens. To further explore the inter-relation between these antigens and cellular differentiation, their development in foetal skin was investigated.

MATERIALS AND METHODS

Skin specimens from twenty-nine human foetuses aged 9–40 weeks (crown–rump length estimates) were cut into 0·5 cm^2 blocks, snap-frozen in a isopentane–liquid nitrogen slurry and stored at -70°C. Within a week of obtaining the tissue, sandwich immunofluorescence staining was performed on frozen sections, 6 μm thick, using standard controls (Nairn, 1969) and high titre pemphigus vulgaris and bullous pemphigoid sera diluted 1:8 in phosphate-buffered saline. Prior to use the pemphigus sera were absorbed with human group AB red blood cells (Szulman, 1960).

The conjugate used to demonstrate the antigen–antibody reaction was fluorescein isothiocyanate (FITC)-conjugated goat antihuman-gammaglobulin with a fluorescein to protein molar ratio of 3·5, and predominant reactivity against IgG. Before use it was absorbed with rat and human tissue homogenates including skin and diluted with phosphate-buffered saline to an approximate concentration of 0·4 g/100 ml so that by itself it gave no staining of the tissue sections studied. Preparations were examined by darkground ultraviolet fluorescence microscopy employing a clear barrier filter and a condenser fitted with a toric lens beneath. Prevention of immunofluorescent staining by neutralization absorptions of pemphigus and bullous pemphigoid sera with human adult and 36 week foetal epidermis confirmed the specificity of the reactions studied.

Skin specimens were also fixed in 10% phosphate-buffered formalin, embedded in paraffin and sections 5 μm thick were stained with haematoxylin and eosin, periodic acid Schiff (PAS) or the silver reticulin stain by the method of Gordon & Sweet (Pearse, 1968).

RESULTS

The development of pemphigus and bullous pemphigoid antigens in human foetal skin is summarized in Table 1. Pemphigus sera gave weak intercellular immunoflourescent staining of the epidermis in the youngest foetuses examined, aged about 9 weeks, the epidermis at this stage being 1–3 cells thick. By 12 weeks, when the epidermis consists of the basal germinative cells, the stratum intermedium, 1–3 cells thick and the outer layer of non-keratinizing periderm (Breathnach & Wyllie, 1965), the intercellular staining had reached the same intensity as in adult skin (Fig. 1). Like the latter, the basal layer of cells in all foetal skins showed little or no intercellular staining.

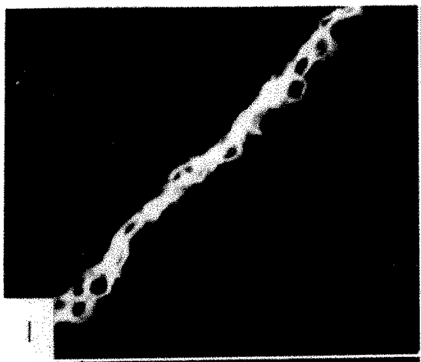
TABLE 1. Pemphigus and bullous pemphigoid antigens in human foetal skin

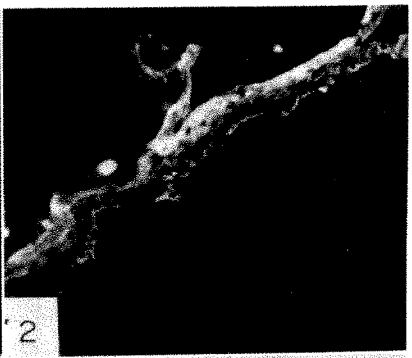
		Intensity of immunofluorescent staining	
Age (weeks)	Number of foetuses	Intercellular	Basement membrane zone
9–10	3	+	–
12–14	4	+++	+
15–20	16	+++	+/++
23–26	4	+++	+++
36–40	2	+++	+++

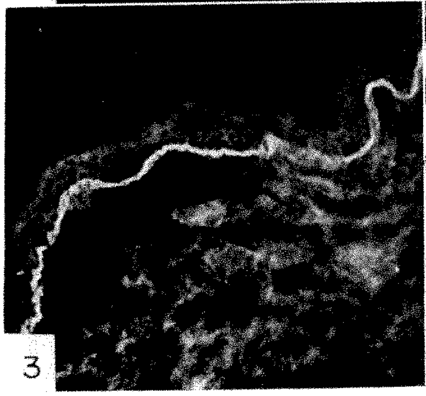
With bullous pemphigoid sera, basement membrane zone staining first appeared at 12 weeks. Initially it was weak (Fig. 2) and sometimes patchy, and although the intensity gradually increased, discontinuity often persisted until about 23 weeks. Whereafter the epidermis, now essentially the same as adult skin (Hashimoto *et al.*, 1966), gave intense linear immunofluorescent staining (Fig. 3). In contrast to the immunofluorescence staining patterns, conventional microscopy, using PAS and reticulin stains, showed the presence of a continuous basement membrane in all foetal skins examined (Fig. 4).

DISCUSSION

The appearance by 9 weeks gestation of the pemphigus antigen in foetal skin precedes the development of a mature keratinizing epidermis. Using peroxidase labelled pemphigus antibody Wolff & Schreiner (1971) have localized the pemphigus antigen ultrastructurally to the intercellular substance and epidermal cell surface. The early appearance of the pemphigus antigen may reflect its functional importance in intercellular cohesion. That intercellular substance rather than desmosomes governs intercellular cohesion is suggested by two observations. Firstly, in pemphigus vulgaris intercellular







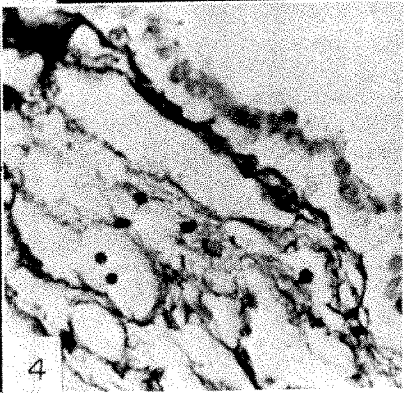


FIGURE 1. Immunofluorescent staining of the intercellular area of 12-week foetal epidermis by pemphigus vulgaris serum (×270).

FIGURE 2. Weak immunofluorescent staining of the basement membrane zone of 16-week foetal epidermis by bullous pemphigoid serum (×180)

FIGURE 3. Bright immunofluorescent staining of the basement membrane zone of 36-week foetal epidermis by bullous pemphigoid serum (×180).

FIGURE 4. Foetal epidermis aged 9 weeks showing well demarcated continuous basement membrane by silver reticulin staining (×390).

cohesion is lost and acantholysis initiated by dissolution of intercellular cement substance, the desmosomes being initially intact (Hashimoto & Lever, 1970). Secondly, treatment of epidermis with cantharidin which disrupts desmosomes and tonofilaments is not accompanied by loss of intercellular cohesion (Wolff, Tappeiner & Schreiner, 1968).

It is possible that loss of the pemphigus antigen reported in squamous cell carcinoma may reflect not so much the degree of cellular differentiation (Muller & Sutherland, 1971), but rather decreased cellular cohesion and hence a greater capacity for tissue invasion. Likewise, studies on healing skin wounds in rats have shown striking correlation between loss of pemphigus antigen and epithelial cell migration, with reappearance of the antigen when epithelialization is complete (Kalnins & Muller, unpublished).

The epidermal basement membrane of skin as defined by ultrastructural techniques is formed in the human embryo by 6 weeks gestation (Breathnach & Robins, 1969). We could not detect the bullous pemphigoid antigen in the basement membrane area before 12 weeks and its immunofluorescent staining pattern was irregular for several weeks thereafter. This lack of correlation of classical basement membrane and bullous pemphigoid antigenicity indicates some degree of independence of the two structures.

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Carbohydrate tolerance in patients with granuloma annulare*

STUDY OF FIFTY-TWO CASES

S.HAIM, R.FRIEDMAN-BIRNBAUM, N.HAIM, A.SHAFRIR
AND A.RAVINA

Department of Dermatology, Rambam Government Hospital and Aba Khoushy School of Medicine.
Diabetes Clinic, Workers Sick Fund of the General Federation of Labour, Haifa, Israel

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SUMMARY

The carbohydrate tolerance was routinely investigated in fifty-two patients with granuloma annulare. Thirteen of the fifty-two cases presented the generalized type and the remaining thirty-nine had the localized form of the disorder. The results obtained in these patients were compared with those revealed in two control groups of patients. The same number of patients of the same sex, age and ethnic group with skin diseases other than granuloma annulare were similarly investigated as were 386 patients with conjunctival micro-aneurysms. Some degree of carbohydrate intolerance was detected in 36·5% of patients with both types of granuloma annulare. However, the incidence of this disturbance in patients with the localized form was no greater than in those with other skin diseases. The important finding in this study was the 76·9% incidence of carbohydrate intolerance in generalized granuloma annulare. This finding was supported by the histopathological changes in the small cutaneous blood vessels of these patients.

Reports dealing with the relationship between granuloma annulare (GA) and diabetes mellitus are conflicting. Some of these reports show no evidence for any such inter-relationship (Wells & Smith, 1963; Dicken, Carrington & Winkelman, 1969; Mobacken, Gisslen & Johannisson, 1970; Williamson & Dykes, 1972). Others indicate that the disease, especially in its atypical generalized form, should be included among those skin disorders known to be associated with the diabetic state (Rhodes, 1966; Romaine, Rudner & Altman, 1968; Haim, Friedman-Birnbaum & Shafrir, 1970; Reinhard *et al.*, 1971; Degos & Guilaine, 1964; Feldman, 1968), such as necrobiosis lipoidica diabeticorum (Muller & Winkelmann, 1966; Hill *et al.*, 1966), diabetic dermopathy (Bauer *et al.*, 1966; Bauer & Levan 1970; Allen & Hadden, 1970; Cantwell & Martz, 1967; Binkley, 1965; Jelinek, 1970) and others.

The contradictory reports suggested to us a routine investigation of the carbohydrate tolerance in patients with GA. We prefer the term 'carbohydrate tolerance' to the term 'diabetes mellitus' since the criteria for the diagnosis of the latter vary from one centre to another.

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*Based on a paper presented before the 14th International Congress of Dermatology, Venice-Padua, 1972.

METHODS

All patients were thoroughly investigated for possible systemic disease. The diagnosis of GA was confirmed, in each case, by histological examination of the skin. In cases with the generalized type of the disease, at least two biopsies from two different sites were performed. These sections were stained also with PAS in order to detect possible vascular changes compatible with those of diabetes.

The carbohydrate tolerance was investigated as follows:

(1) In cases with an obvious history of diabetes, the fasting glucose level in the blood was determined.

(2) In patients with no history of diabetes, the prednisone glucose tolerance test was done.

The test was performed after 3 days on a diet of 300 g of carbohydrate daily, by the oral administration of 10 mg prednisone 8 and 2 h before a standard oral glucose tolerance test.

(3) The glucose level was determined by Hagedorn–Jensen's method.

(4) Levels of glucose above 180 mg/100 ml at the end of the first hour of the test and above 160 mg/100 ml after 2 h were considered pathological. These figures correspond to the pathological values given by Fajan (1963), with the addition of 20 mg since the maximal normal glucose level according to our laboratory is 120 instead of 100 mg/100 ml.

(5) Doubtful cases and cases with a rise of glucose level above 180 mg/100 ml at the end of the first hour of the test and return to normal during the second hour were further investigated. The rate of disposal of glucose (*k*) was determined by the intravenous glucose tolerance test and the insulinogenic reserve by the tolbutamide test.

The value of *k* was considered to be 1·15 (Camerini-Delavos *et al.*, 1962). The tolbutamide test was performed by the intravenous injection of 1 g tolbutamide. A reduction of 23% or more of the fasting initial level of glucose indicated a normal reserve.

The results of the above investigations were compared with those obtained in two control groups:

(1) The same number of patients with other skin diseases unrelated to diabetes was similarly investigated. The patients were not known to be diabetics and were of the same sex, age and ethnic group as those with GA.

(2) 386 patients with conjunctival micro-anuerysms who underwent a similar routine prednisone glucose tolerance test in another medical centre (Wolfsohn & Lavyel, 1967).

CLINICAL MATERIAL

Fifty-two patients with GA were investigated, fourteen male and thirty-eight female. Their average age was 42·3 years: the youngest a girl aged 5 years, and the eldest a 72-year-old woman. Thirteen of the fifty-two presented the generalized type of the disease. It was manifested by a symmetrically distributed eruption involving mainly the sun-exposed areas of the body, and consisting of small papules, discrete, or confluent to produce plaques or annular and reticulate configurations. These patients were mostly of eastern european origin and consisted of two males and eleven females. Their average age was 53·3 years. The remaining thirty-nine, suffering from the localized type, presented with 1–5 annular lesions. There were twelve males and twenty-seven females with an average age of 38·6 years.

Table 1 shows the age distribution of those patients. Generalized GA is found in the middle and upper age groups. Localized GA has a much wider age distribution.

RESULTS

Seven of the fifty-two patients with GA, four of the localized type and three of the generalized type, were known diabetics. Thirteen with localized GA and four with generalized GA had a family history of diabetes mellitus. Physical examinaton revealed no pathology besides their skin disease and apart from those of the carbohydrate disorder, there were no pertinent laboratory findings.

Nineteen (36·5%) of these patients showed intolerance to carbohydrate. Nine (23·1%) of the thirty-nine with localized GA and ten (76·9%) of the thirteen with the generalized type. This difference in frequency between the two types of the disease was not related to the age incidence and is evident when matched for age (Table 2).

TABLE 1. Age distribution

Age (years)	1–10	11–20	21–30	31–40	41–50	51–60	61–up	Total
Generalized GA	–	–	–	1	3	6	3	13
Localized GA	2	6	7	2	9	10	3	39
Total	2	6	7	3	12	16	6	52

TABLE 2. Incidence of carbohydrate intolerance

Age (years)	1–10	11–20	21–30	31–40	41–50	51–60	61–up	Total	%
Localized GA	0(2)*	1(6)	0(7)	0(2)	3(9)	3(10)	2(3)	9(39)	23·1
Generalized GA	–	–	–	0(1)	3(3)	4(6)	3(3)	10(13)	76·9
Total	–	1	–	–	6	7	5	19(52)	36·5

* Figures in brackets represent total number of patients examined in the same age group.

The histological study of PAS stained sections showed a coarse and hyalinized basement membrane of the capillary walls due to deposition of an amorphous, eosinophilic, PAS-positive, material in all the thirteen cases with generalized GA, including the three who were biochemically normal. In the localized GA group, these changes were demonstrated only in those who were biochemically abnormal.

In the control group, the incidence of pathological glucose tolerance curves was 23%, i.e twelve of the fifty-two patients who were similarly investigated.

In the group of patients with conjunctival micro-aneurysms only, the prednisone glucose tolerance test was abnormal in forty-eight (11·67%) of the 386 cases, and doubtful in a further thirty-eight (9·8%). The frequency of an abnormal prednisone tolerance curve in these ranged from none in the age groups up to 30 years to 23·3% at the ages of 60–70.

DISCUSSION

The term carbohydrate intolerance has been used in preference to 'diabetic curve', in order to avoid invalid conclusions. While the standardization of the tests performed in not above criticism, it is nevertheless felt that the conclusions of this work are based on results obtained using identical standards for the patients and the control groups.

Rhodes *et al.* (1966) demonstrated an abnormal prednisone glucosuria test in one-third of thirty patients with GA. Wood & Beerman (1960) studied the thickness of capillary walls in relation to the diameter of the lumen and reported moderate thickening in some cases of GA but always less than in necrobiosis lipoidica diabeticorum. In our group of patients with GA, carbohydrate intolerance and the vascular changes were both present in 36·5% of the cases. However, a further analysis of our results shows that the localized GA seems not to have a different incidence of carbohydrate intolerance than other skin diseases (23·1 and 23% respectively), as compared with an incidence of 76·9% in the generalized GA group (Table 3).

TABLE 3. Summary of results

Group of patients	No. of cases	% doubtful	% abnormal CHO tolerance
Localized GA	39	–	23·1
Generalized GA	13	–	76·9
Other skin disease	52	–	23·0
Conjunctival micro-aneurysms	396	9·8	11·67

It is of interest to note that the incidence of carbohydrate intolerance in the patients with conjunctival micro-aneurysms which are considered by some (Wolfsohn & Lavyel, 1967) to be a feature of diabetes, was only 11·67% with a further 9·8% doubtful. Our data, therefore, permit us to speculate that as far as carbohydrate metabolism is concerned the two forms of GA seem to differ.

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Familial hyperkeratosis lenticularis perstans associated with tumours of the skin

G.W.BEVERIDGE AND A.O.LANGLANDS

Department of Dermatology, The Royal Infirmary, Edinburgh, and Department of Radiotherapy, The Royal Infirmary and Western General Hospital, Edinburgh

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SUMMARY

A pedigree from a family with hyperkeratosis lenticularis perstans which shows a pattern of dominant inheritance is presented. This would appear to be the second occasion in which this condition has been shown to have a genetic basis. This family in addition shows a high incidence of cutaneous carcinomata, not arising in the keratosis. The possible significance of the association is discussed.

Since the first report by Flegel (1958) of punctate keratoses characteristically affecting the extensor surfaces of the legs and the dorsa of the feet, several other reports have confirmed this as a distinct clinical entity (Gruneberg, 1963; Hunter & Donald, 1968; Kocsard, Bear & Constance, 1968; Raffle & Rogers, 1969). The name hyperkeratosis lenticularis pertans (HLP) has been proposed for this condition. In 1969 Bean reported, for the first time, the familial occurrence of the disease, apparently inherited as a dominant trait.

The present family is reported for two reasons: because we believe it to be a second example of the familial inheritance of HLP, and because in this family there was also a tendency to develop tumours of the skin.

CASE REPORTS

The full family pedigree is shown in Fig. 1.

Case I (III, 5)

The propositus presented at the age of 50 with a histologically proven basal cell carcinoma of the right side of the nose. Seventeen years earlier a cutaneous horn had been removed from the left arm.

The basal cell carcinoma responded satisfactorily to superficial X-ray therapy and a year later a second basal cell carcinoma was excised from below the left eyelid. When he was 57 a third basal cell carcinoma in the right nasolabial fold was treated by X-ray therapy. At the time of treatment of the third basal cell carcinoma a positive family history was obtained and other members of the family were examined. He has since had excised from the left side of the neck a keratotic nodule which showed the features of a keratosis with possible early squamous carcinomatous change.

He had X-ray therapy for ankylosing spondylitis when aged 54 years. His only other significant medical history was of chronic peptic ulceration.

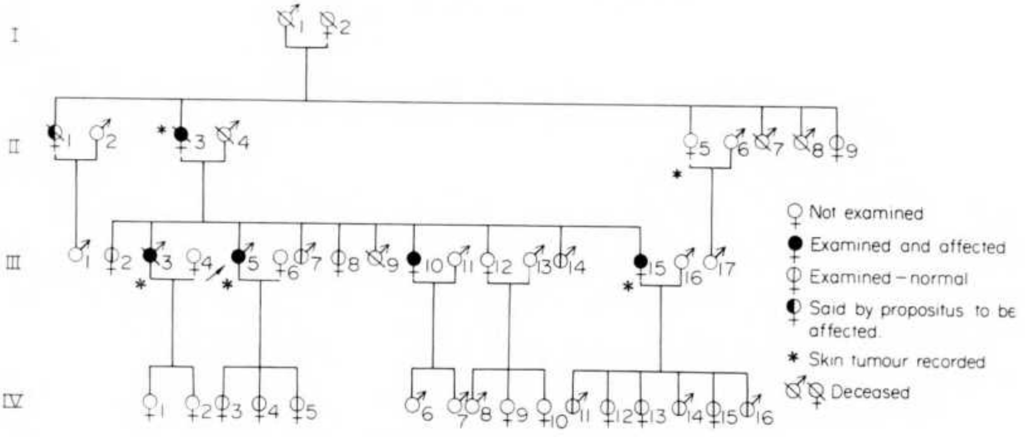

FIGURE 1. Family pedigree.

The striking features on clinical examination were of marked telangiectasia of the exposed areas of face and neck and the presence of discrete areas of hyperkeratosis on the dorsa of the feet (Fig. 2) and similar lesions on the posterior aspects of the lower legs and on the ulnar borders of the hands. These keratotic lesions had been present from about the age of 40. There was slight erythema at the margin of the keratosis but no marked infiltration. The keratoses were firmly adherent to the skin but the patient had noticed that occasionally they became detached but tended to recur. A typical lesion was biopsied and showed dense hyperkeratosis on a slightly acanthotic epidermis and a moderate chronic inflammatory infiltrate in the upper dermis, which is consistent with a diagnosis of HLP.

Case 2 (III, 3)

At the age of 4 this patient's right eye was enucleated and at the age of 29 the left eye, the second operation being carried out for 'buphthalmos'. When he was 44 he presented with a 'warty lesion'

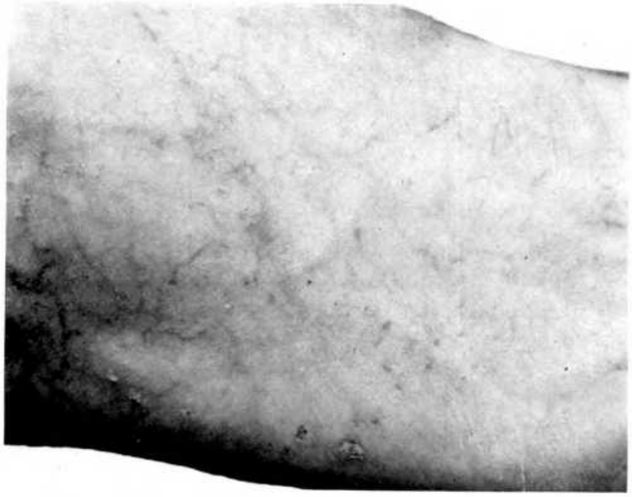

FIGURE 2. Clinical appearance of keratoses, Case 1 (III, 5).

at the junction of the right ear and face. This was excised and the specimen was reported as showing the features of a well differentiated squamous cell carcinoma.

He remained well thereafter and was examined by us when aged 60. He was found to have hyperkeratotic lesions of the legs and dorsa of the feet identical to those occurring in his brother.

Two years later, he was found to have a peripheral carcinoma of the left lung for which a left upper lobectomy was carried out. He died within the post-operative period. Histological examination of the lung tumour showed it to be an anaplastic carcinoma.

Case 3 (III, 10)

This patient was examined when aged 45 and at that time had comparable hyperkeratotic lesions on the legs and feet. She had no other significant medical history.

Case 4 (III, 15)

At the age of 24 this patient presented with an ulcerated lesion of the right forehead. This was excised and histologically showed changes of a well differentiated squamous cell carcinoma.

When examined at the age of 37, she had characteristic hyperkeratotic lesions on the lower legs and the dorsa of the feet. In 1969 a typical hyperkeratotic lesion was excised and showed the features of HLP. A nodular lesion from the back of the left lower leg which had progressively enlarged was excised and histologically this was a well differentiated squamous cell carcinoma.

Case 5 (II, 3)

This patient was the mother of the first four cases. Between the age of 50 until her death, aged 78, in 1967, this lady had at least twelve lesions excised from the skin of head and neck. Histological material from six of these lesions was traced and was reported as benign squamous proliferation, cutaneous horn and on three occasions squamous cell carcinoma.

The presence of hyperkeratotic lesions on the flanks and legs which came and went were recorded on more than one occasion in this patient's clinical records. One lesion, biopsied when the patient was 59, has been re-examined, and is consistent histologically with a diagnosis of HLP.

REVIEW OF PATHOLOGY

Biopsies of representative hyperkeratotic lesions were obtained from Cases 1 and 4 and it was also possible to trace a biopsy taken in 1949 from the leg of Case 5 and to examine sections from this. In all three instances the histological features were similar to those described by Flegel. On the surface there was a dense laminated plaque of keratin which showed patchy parakeratosis but there were no parakeratotic columns. At the margins of the lesions the epidermis was acanthotic. In the sections from Cases 1 and 5 there was only a very shallow depression of the epidermis whilst in the section from Case 4 there were two areas of depression. The two former sections showed no evidence of penetration into the dermis but in the lesion in Case 4 penetration occurred into the upper dermis. The epidermis at the base of the lesions was thinned but still exhibited a normal granular cell layer. The keratinocytes showed no atypical features and the dermo-epidermal junction was intact. In the upper dermis there was a chronic inflammatory infiltrate which consisted mainly of lymphocytes.

Sections from as many tumours as possible reported in the clinical case histories have been obtained and re-examined.

Case 1 (III, 5)

Three of the lesions submitted for histology from this patient's face showed the features of basal cell carcinomata. No histology was available from the cutaneous horn removed from his left arm, but the lesion from the left side of neck showed features of hyperkeratosis on a proliferative epidermis and in one area there was disorganization of the keratinocytes with some dyskeratotic cells which suggested early nest formation. It was considered to be only pre-malignant.

Case 2 (III, 3)
This section unfortunately had been traumatized so that interpretation was difficult because of distortion and nuclear damage. The appearances were suggestive of a well differentiated squamous cell carcinoma.

Case 4 (III, 15)
The histology of the lesion on the forehead was reviewed. The epidermis throughout the section showed variation in thickness. In some areas at the mouths of hair follicles, there was heaping of keratin. In one area the epidermis shows proliferative changes and penetration of squamous cells into the upper dermis where there was evidence of whorl formation and central keratinization. This was again in keeping with the diagnosis of a well differentiated squamous carcinoma.

Case 5 (II, 3)
Sections were obtained from lesions removed at varying times during the patient's life. In four of these there is no doubt that the lesion was a well differentiated squamous carcinoma.

Case 6 (II, 5)
The sections showed invasion by a moderately differentiated squamous carcinoma.

In summary, most of the tumours regarded as malignant were well differentiated squamous cell carcinomata. Although it is always difficult on histological grounds to differentiate this type of change from pseudo-epitheliomatous change, we felt on reviewing the sections they could be regarded as showing low grade malignancy. Further support is given to this view by the fact that most of the tumours were removed previously by a number of surgeons who clinically regarded the lesion as malignant and treated them by radical excision and in the case of the tumour on the forehead of III, 15, excision was followed by grafting of the area.

DISCUSSION

The five cases which have been examined and the remaining case described by relatives (Fig. 1) all had similar lesions on the lower limbs. These lesions which appeared in the third and fourth decades were discrete areas of hyperkeratosis usually measuring only a few millimetres in diameter. They were firmly adherent to the skin, and around their margins there was slight redness and minimal infiltration. All these features and the histology were similar to those described in hyperkeratosis lenticularis perstans.

The possibility of acquired keratoses as a response to a common environmental factor was considered. There was no evidence of arsenic ingestion and the lesions histologially showed none of the epidermal features of arsenical keratoses, namely irregular epidermal proliferation with dyskeratotic and Bowenoid cells within the epidermis. The keratoses were not on the areas maximally exposed to light and again histologically there were none of the histological features such as atypical or dyskeratotic keratinocytes commonly seen in solar keratoses.

Other conditions which have a genetic basis and may be characterized by hyperkeratotic lesions were considered. Darier's disease, acrokeratosis verruciformis and porokeratosis of Mibelli were quickly excluded on clinical and histological grounds.

Finally, Prakken (1954) observed Kyrle's disease in siblings, and although a genetic background for this disease has rarely been recorded, on clinical grounds the diagnosis was initially considered. Wilson-Jones & Garcia e Silva (1972) have postulated a possible relationship between Kyrle's disease and HLP as cases with clinical and histological features intermediate between the two syndromes are not infrequent. The lesions in our patients are considered to conform most nearly to the HLP end of the clinicopathological spectrum.

There is evidence from the family tree (Fig. 1) that the condition is dominantly inherited and thus this would appear to be the second report of the familial incidence of this condition, first noted by Bean in 1969 when he reported three cases in one family. Since the father and two siblings were also known to be affected, Bean suggested that the condition was transmitted as an autosomal dominant. Subsequently Bean (1972) has reported the development of HLP in a further member of this family which indicates penetration through three generations. No member of generation IV examined by us showed evidence of HLP. It should be pointed out, however, that of those examined, the eldest was only 28. The hyperkeratotic lesions appeared in the affected individuals in the third or fourth decades and members of generation IV were still too young to exhibit the trait at the present time.

The second feature of considerable interest in this family is the high incidence of skin tumours. These were mainly squamous cell carcinomata but the propositus also had three histologically proven basal cell carcinimata. Histology was not available from all the tumours which showed squamous proliferation, but sections from eight tumours from five individuals were examined and all showed the features of a well differentiated low grade squamous carcinoma. The tumours with one exception, namely the lesion from III, 15 occurred on sites other than the areas affected by HLP. In addition, two of the cases presented died from carcinoma of the lung (III, 3 and II, 3) as did the maternal grandfather (I, 1) of the propositus. On the basis of the high incidence of skin tumours in this family and also possibly the other epithelial carcinomata noted it is suggested that they may also have in addition to HLP an inherited tendency to epithelial neoplasia. There would appear to be no direct connection between the neoplastic lesions and the keratotic lesions since, with one exception, they occurred in different sites. On the present evidence it may be that the association with epithelial neoplasia in this family is fortuitons. It was felt, however, worth reporting this since Flegel's disease has only been recently recognized to be familial and if this association is significant further families may be reported in the future.

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The cutaneous barrier to salts and water in psoriasis and in normal skin

KATHERINE GRICE, H.SATTAR AND HARVEY BAKER

The London Hospital, Dermatology Department,
Whitechapel, London E.1

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SUMMARY

A study has been made of the transepidermal water loss and its sodium and potassium content in normal and psoriatic skin. The results show a preservation of the electrolyte barrier in active psoriasis despite serious impairment of the water barrier.

In normal skin the cutaneous barrier effectively prevents significant loss of water (in the absence of sweating). Passive transepidermal water loss is in the range of 0·2–0·7 mg $cm^{-2}h^{-1}$ (Pinson, 1942; Bettley & Grice, 1965; Baker & Kligman, 1967; Johnson & Shuster, 1969b; Shahidullah *et al.*, 1969; Spruit & Malten, 1971). There are fewer data on electrolyte loss in epidermal transudate. Hancock, Whitehouse & Haldane (1929) found that the amount of salt on the surface of the skin was much less than should have been present were water loss due solely to sweating. They therefore deduced that water was lost from the skin by two methods: (a) sweating which had a high salt content; (b) filtration through the skin of water containing negligible salt.

Measurement of the galvanic skin resistance may give an indication of the salt content of the epidermal transudate. The electrical resistance of normal skin to the passage of a small current is high in the absence of sweating, and drops sharply when sweating takes place (McPherson & Malcolm, 1951; Kuno, 1956). In eczema (Gougerot, 1947) or in contact dermatitis in the absence of sweating (Bettley & Grice, 1966) the skin resistance is low suggesting that the increased epidermal transudate might contain appreciable salt.

In psoriasis transepidermal water loss is greatly increased to between 2.0 and 6·0 mg $cm^{-2}h^{-1}$ (Felsher & Rothman, 1945; Grice & Bettley 1967; Baker, 1971) indicating serious impairment of the cutaneous water barrier. However, the finding that the galvanic skin resistance was extremely high in some psoriatic lesions despite a large transepidermal water loss suggested that the electrolyte content of the transudate might be very low (Grice, 1967).

We have therefore measured directly sodium and potassium levels in epidermal transudate in normal and psoriatic skin *in vivo.*

METHOD

Sweating was inhibited on 120 cm^2 of the anterior surface of the forearm in fifteen normal subjects by painting with 4% poldine methosulphate in 70% ethyl alcohol and occluding overnight with polythene wrap. The next morning, the polythene was removed and 4–5 h later the galvanic skin resistance measured to ensure that sweating had been abolished. The apparatus used for measuring the skin resistance was similar to that previously described (Bettley & Grice, 1965). The forearm was washed thoroughly with soap and water, rinsed well with tap water and then with deionized distilled water and air dried. Whatman 540 filter papers 11·0 cm diameter were weighed in airtight polythene bags. To ensure that the skin had been adequately cleaned, three filter papers dampened with 0·5 ml deionized distilled water were placed in turn on the forearm under cover of polythene that had itself previously been washed, rinsed in deionized distilled water, and dried. The skin was mopped dry and the damp papers were followed by pre-weighed dry filter papers. The dry filter papers were replaced in their containers and re-weighed. These procedures were to ensure adequate skin cleansing.

The dry pre-weighed paper was then placed in position and covered with polythene the same shape as the filter paper, but a few millimetres larger. The edges were sealed with waterproof adhesive plaster and the filter paper left in place for 6 h. At the end of the test period, the filter paper was re-weighed. Sodium and potassium content of the filter papers were analysed by flame photometry.

The same procedure was used in six subjects with active psoriasis. They all had discoid plaques larger than the size of the filter paper. Topical treatment had been omitted for 24 h previously. In view of the higher transepidermal water loss through psoriatic skin, the filter paper was left in position for only 3 h. Of the filter papers available, Whatman 540 contained the least sodium. The amount present was appreciable however and was deducted from the sodium determinations in the transepidermal fluid. The method for cleaning the skin was satisfactory as the two final papers (one damp and one dry) before the test period contained no appreciable sodium or potassium. Systemic absorption of the anti-cholinergic drug was sufficient to cause a dry mouth in two of the subjects with psoriasis.

The transepidermal water loss was measured and corrected for skin temperature as described previously (Grice *et al.*, 1971).

TABLE 1. Normal skin. Sodium and potassium concentration in epidermal transudate in fifteen cases. Galvanic skin resistance > 10^9 Ω in all cases

Case no.	Sodium concentration (mmol/l)	Potassium concentration (mmol/l)
1	4·2	0·7
2	9·6	0
3	8·9	1·4
4	8·5	1·8
5	7·1	1·8
6	2·5	0
7	5·5	0·8
8	7·7	1·1
9	6·7	0
10	9·5	0·8
11	4·8	1·2
12	10·3	1·7
13	5·5	0·8
14	7·1	0
15	11·0	2·4
Mean	7·2 ± 2·4	0·9 ± 0·7

RESULTS

Normal subjects ($n = 15$)

The mean transepidermal water loss (TEWL) in fifteen normal subjects was $0{\cdot}3 \pm 0.1$ mg cm^{-2}h^{-1} (corrected to skin temperature 30°C). The sodium and potassium content in the epidermal transudate is shown in Table 1. The mean sodium concentration was $7{\cdot}2 \pm 2{\cdot}4$ mmol/l and the mean potassium concentration $0{\cdot}9 \pm 0{\cdot}7$ mmol/l. In all fifteen, the galvanic skin resistance was greater than $10^9 \Omega$ at the time of measurement.

Psoriatic subjects ($n = 6$)

The TEWL range was from 2·0 to 5·6 mg cm^{-2}h^{-1}. The sodium and potassium concentrations in the epidermal transudate are shown in Table 2. Five of the six patients had sodium levels of 9·0 mmol/l or less. In only one patient (discoid psoriasis) was the sodium content outside the normal range (24·4 mmol/l) and, as expected, the galvanic skin resistance was lower in this patient than in the rest. In the others it was more than $10^9 \Omega$.

The potassium concentrations were within the normal range in all six patients (mean 0·8 mmol/l, range 0–2·1).

TABLE 2. Psoriasis. Sodium and potassium concentration of epidermal transudate and galvanic skin resistance (SR)

Case no.	Sodium concentration (mmol/l)	Potassium concentration (mmol/l)	SR ($10^8 \Omega$)
1	9·0	1·4	> 10
2	4·1	0·3	> 10
3	3·2	0	> 10
4	8·0	1·0	> 10
5	2·3	0	> 10
6	24·4	2·1	5–10
Mean	8·5	0·84	

DISCUSSION

In subjects with normal skin, transepidermal water loss assumes little importance in terms of metabolic balance. In generalized exfoliative dermatitis, the deficient water barrier is of metabolic importance, the skin losing up to 2·0 l or more of water per day by the transepidermal route.

From the present studies the salt content of the epidermal transudate is likely to be low in psoriasis and therefore needs replacement by water with a low salt content. Sweat loss in normal skin may be as high as 3 l in 4 h (Macpherson, 1960) but sweating in eczema or psoriasis is diminished or absent (Cormia & Kuykendall, 1955). The average sodium content of sweat is usually taken as being 60 mmol/l and that of potassium 8 mmol/l, but the concentrations vary greatly depending on factors such as sweat rate, hormonal control, salt diet, etc. (Dobson & Sato, 1972). The overall total impact of widespread psoriasis on sodium and potassium balance is being investigated using the resources of a metabolic ward as an extension of these studies.

Were the skin barrier completely destroyed one would expect the salt content of the epidermal transudate to be similar to that of the extracellular fluid, i.e. sodium being 142 mmol/l and potassium 5 mmol/l (Guyton, 1971). We found sharp falls of skin resistance (in spite of absence of sweating) in some areas of psoriasis. This suggests, as one would expect, that the epidermal disorder is not entirely uniform in psoriasis, some areas being sufficiently disturbed functionally to allow both increased salt and water passage through the skin.

In normal skin with an intact barrier, the salt content of epidermal transudate was found to be very low. The opinion is generally held that the stratum corneum acts as a passive porous mechanical barrier and the *in vivo* and *in vitro* rates of water filtration through human skin are similar (Scheuplein & Blank, 1971). Two methods of filtration through membranes have been described. Various substances either dissolve in the lipid of the membrane or they pass through minute pores or channels in the membrane (Guyton, 1971). In mammalian skin, unlike frog's skin (Bonting & Caravaggio, 1963; Ussing, 1965; Davis, 1969) there is no evidence of active transport of sodium (Tregear, 1966a). The penetration of electrolytes through human skin in either direction is poor; Tregear (1966b) showed that the *in vivo* absorption rate of sodium from the human skin surface was similar to its *in vitro* penetration rate.

We found that the sodium and potassium concentrations in the epidermal transudate in psoriasis were very low, the potassium and four of the five sodium levels being within the range found in transudate from normal skin. The difficulty of cleaning the skin adequately could mean that the salt content of the epidermal transudate was even lower. Johnson & Shuster (1969a) showed that the sodium and potassium contribution from scales was likely to be negligible.

The present investigations therefore show that in psoriasis, although there may be damage of the barrier to water filtration through the skin, the concentrations of sodium and potassium are usually normal. This finding could be explained by a theoretical increase in the number of membrane 'pores' or channels available in the skin barrier in psoriasis, the pores still being too small for the hydration spheres around the ions to pass through. In those areas where there was an increase in the salt content of the epidermal transudate it could be argued that the diameter of the membrane pores had increased.

If there were an active transport system for sodium and potassium in human skin, our data would suggest that this system remains intact in psoriasis although the passive leakage of water through the skin continues.

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Iron in finger-nails and in the liver

L.L.CHANG, C.L.GOH AND G.R.WADSWORTH

Department of Physiology, University of Singapore

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SUMMARY

The mean iron content of finger-nails of 113 apparently normal subjects was found to be 67·6 μg/g dry nail (±43·7). There was no age or sex variation. No relationship was found between the concentration of iron in finger-nails and that stored in the liver.

The total amount of iron in the body is thought to vary from about 280 mg in a newborn infant, to about 3–4 g in a healthy adult (Bothwell & Finch, 1962). Of the total, about 65% is in haemoglobin, about 30% is stored in the liver, spleen and bone marrow and the rest is in myoglobin, enzymes and plasma (Bothwell & Finch, 1962). An assessment of the quantity of stored iron is useful since latent iron deficiency has been claimed to exist with a reduced amount of iron in the stores, but with normal red blood cell morphology, haemoglobin concentration, packed cell volume, serum iron concentration and total iron binding capacity (Weinfeld, 1965). Assessments of the amount of iron in the stores can be made from staining bone marrow smears for haemosiderin (Rath & Finch, 1948), observing the effects of phlebotomy (Hynes, 1949: Haskin *et al.*, 1952) or measuring the excretion of iron after its chelation in the body with desferrioxamine (Gale, Torrance & Bothwell, 1963). None of these methods is a direct measure of the amount of iron which is stored, and their application is usually not justifiable in the absence of overt signs or symptoms of anaemia. Moreover, the interpretation of the results from children is complicated by normal variation of the distribution of iron in the body during growth (McCance & Widdowson, 1951). In the search for a simple and innocuous method for assessing the state of the body's iron stores a study was made of the iron content of finger-nails and its relationship to the amount of non-haem iron in liver.

MATERIAL AND METHODS

Clippings were made from the free edges of finger-nails of people of different ages. In adults a single clipping was sufficient for duplicate estimations of iron concentration, but in children the nails from two or three clippings were required. A total of 113 subjects was studied. All were apparently normal and nearly all were Chinese. Finger-nail clippings and portions of the liver were also obtained at autopsy on thirty-five people who had died from accidents.

The nails were washed and dried prior to analysis by the method described by Jacobs & Jenkins (1960). Samples of approximately 40 mg of dried nail were used for estimation of iron by an adaptation of a method described by Wootton (1958). The samples were digested in a conical flask over a hot plate with 2 ml of a mixture of perchloric acid and concentrated nitric acid (3:5) until a white residue was obtained. This was dissolved in 0·5 ml perchloric acid and 2·0 ml iron-free water. The optical density of the ferric perchlorate solution so obtained was read in a Unicam SP500 spectrophotometer at a wavelength of 240 mμm against a blank consisting of the acid digestion mixture treated in the same way as the test material. A calibration curve was constructed using a solution containing 2–10 μg iron made from dilutions of a standard preparation (BDH) and treated in a similar manner as the samples. Results were expressed as μg/g dry nail.

The amount or iron stored in the liver was estimated by the method of Kaldor, (1954) using *o*-phenanthroline. Results were expressed as μg/g wet liver.

RESULTS

The mean iron contents of finger nails for the different age groups are shown in Table 1. There was no significant difference, as revealed by 'Student's' *t* test, between the concentrations in the nails from subjects of different age groups. The overall mean content of iron was 67·6 μg/g dry nail (±43·7). There was a wide scatter of the results within the different age groups.

Results from the thirty-five autopsy specimens are shown in Fig. 1. There was no significant

TABLE 1. Concentration of iron in finger-nails according to age

Age (years)	Number	Iron content (μg/g dry nail) ±1 SD
<10	19	88·9±45·4
11–20	25	77·2±53·8
21–30	20	53·3±41·8
31–40	10	68·2±15·8
41–50	9	51·4±40·6
51–60	18	46·5±28·1
>61	12	81·2±40·4
Mean	113	67·6±43·7

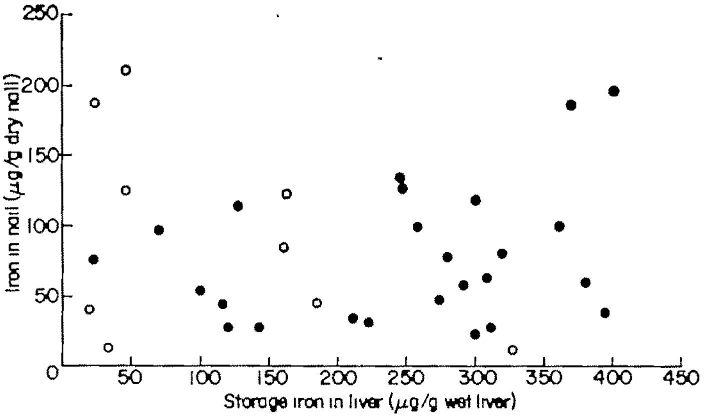

FIGURE 1. The relationship between nail iron and liver storage iron. ● male; ○ female.

correlation between the concentrations of iron in finger-nail and in the liver (correlation coefficient $= -0{\cdot}03$).

DISCUSSION

There is no standard method for estimating the iron content of nails. Of late, atomic absorption spectroscopy has been used more frequently than other methods. The lack of standardization of methods and the absence of a simple and satisfactory procedure for cleaning nail samples prior to analysis has led to difficulties in comparing the results from different studies. The method used in the present investigation was an adaptation of one described by Wootton (1953) for the measurement of the iron content of biological materials. The procedure is easy and only requires by way of specialized equipment a spectrophotometer capable of reading in the ultraviolet range.

Table 2 compares the results obtained in the present series with those by other recent investigators.

The present results, although somewhat higher, were of the same order as those found by Vellar (1970) and Harrison & Tyree (1971). Jacobs & Jenkins (1960) obtained much higher values. The reason for this discrepancy is not apparent, but if technical factors can be discounted, it suggests that people in Britian have more iron in their bodies than do people in some other parts of the world. Jacobs & Jenkins (1960) also found that the concentration of iron in the nails of young children was comparatively high. The present results, although limited in this respect, did not show such a pattern.

TABLE 2. Comparison of results of the measurement of iron in nails by different investigators.

Author	Number	Mean	Range
Jacobs & Jenkins (1960)	100 (both sexes)	346·8	–
Vellar (1970)	12 (both sexes)	27·0	–
Harrison & Tyree (1971)	7 (females)	38·0	14–90
	10 (males)	41·0	28–109
Present study	51 (females)	69·5	11·4–210·0
	62 (males)	66·1	10·0–195·5

Nails are formed by the keratinization of epidermal cells and hence may reflect the iron content of the epidermis. Hussain & Patwardhan (1959) showed that the iron content of sweat, which is contributed mainly by desquamated epidermal cells, rises and falls with corresponding changes in the amount of iron in the body. They showed that patients with iron deficiency anaemia had little iron in their sweat; the amount increased after treatment with iron. Jacobs & Jenkins (1960) reported that the iron content of the nails of their anaemic patients who were less than 20 years, or more than 50 years, of age were lower than the mean value of normals at those ages. They could not demonstrate any relationship, however, between the level of haemoglobin and the iron content of the nails. Lovric & Pepper (1971) reported the absence of a relationship between hemoglobin levels and the iron content of hair.

No previous study has been made to relate finger-nail iron with the state of the iron stores. Iron-containing enzymes contribute to the iron found in nails. Reductions in iron-containing enzymes are thought to accompany falls in storage iron and are considered to play an important role in the widespread tissue defects seen in such conditions (Beutler, 1964). However, we did not find evidence of an association between iron deficiency and the amount of iron in nails. The finger-nail iron contents in a few subjects who had low liver storage iron concentration was no different from those with normal iron stores. This may have been due to the fact that whereas iron in enzymes in actively metabolizing cells and in the body stores is labile, that in the nails is probably outside the metabolic pool.

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Effect of cytochalasin B on epidermal cell migration *in vitro*[*]

T. NISHIKAWA[†] AND R. MARKS

Department of Pathology and Institute of Dermatology,
St John's Hospital for Diseases of the Skin,
Leicester Square, London W.C.2

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SUMMARY

Epidermal cell migration occurs at the edges of human skin fragments when these are placed in a suitable medium containing serum. In this investigation, in which seventy-four skin explants were used, it was found that cytochalasin B reversibly inhibited this migratory process. Inhibition was observed at concentrations of greater than 2 μg/ml, and only in a small proportion of explants cultured in the presence of cytochalasin at a concentration of 1 μg/ml. The reversible inhibition of cell movement by cytochalasin B has been thought due to the disruption of cytoplasmic microfilaments and it is possible that this is the mode of action of this substance on the epidermis as well.

During organ culture of adult human skin, epidermis migrates from the cut edges around the dermal aspect of the skin fragment and this *in vitro* migratory process has been termed 'epiboly' (Medawar, 1948; Sarkany, Grice & Caron, 1965; Cruickshank, 1965; Marks, Bhogal & Dawber, 1972). We believe that this migratory process is analogous in some respects to the epidermal cell migration that takes place after epidermal injury, constituting the earliest phase of wound healing.

The cytochalasins are a group of compounds obtained from fungi which have the property of reversibly inhibiting movement of many types of cells by interfering with a system of cytoplasmic microfilaments (Carter, 1967; Wessels *et al.*, 1971). One of these compounds termed cytochalasin B has been used in this investigation to determine whether the cell movement comprising epiboly was susceptible to this inhibitory action.

MATERIALS AND METHODS

Normal human skin was obtained with the Castroviejo keratotome (set at 0·4 mm) from the lateral aspect of the thighs of volunteers who had given their informed consent. Explants measuring 0·5 cm square were prepared and floated epidermis upwards in Eagle's minimal essential medium (Wellcome Research Lab. Kent) supplemented with 10% pooled normal human serum. Seventy-four explants were used in the present study. The details of the culture procedure are given elsewhere (Marks *et al.*,

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[*] Based on a paper read at the Annual Meeting of the British Association of Dermatology, July 1972.

[†] Present address: Department of Dermatology, Keio University School of Medicine, Tokyo 160, Japan.

1972); culture was maintained for periods of either 48 or 72 h. After culture the explants were 'snap frozen' in liquid nitrogen and sectioned at 8 μm on a cryostat, or fixed in 10% formalin, dehydrated and embedded in paraffin before sectioning.

The degree of epidermal migration in each explant was estimated after inspection of at least five separate sections and recorded on an arbitrary scale ranging from 0 to 3, the last point representing almost total or total encirclement by the epidermis and the first representing the earliest signs of epiboly. This arbitrary method has been found to correlate well with the assessment of the degree of migration when the entire tissue has been sectioned. Cytochalasin B (ICI Research Lab.) was dissolved in absolute alcohol and added to the culture media so that final concentrations were attained in the medium ranging from 2×10^{-5} to 2 μg/ml. No culture contained more than 0·2% of alcohol and the same quantities were also added to control cultures. To test the reversibility of the effect of cytochalasin B, in some experiments, four explants were placed in each Petri dish and one of each pair was removed at the end of 24 or 30 h culture and prepared for histological examination. The other two were washed in plain media and cultured for additional periods of 18 and 42 h in serum supplemented media.

In fourteen cultures tritiated thymidine was added to the medium in a concentration of 1 μCi/ml (Radioactive Chemicals, Amersham, specific activity 26 Ci/mmol, Batch No. 18) in the last 4 h of culture so as to identify autoradiographically those cells in DNA synthesis. Autoradiographs were prepared using the dipping method and Ilford nuclear emulsion K2. Exposure times were for 2 and 3 weeks.

RESULTS

Control cultures

All the control explants showed some degree of epidermal migration and were in a good state of preservation as judged by their histological appearance and the ability of those cultures tested to incorporate tritiated thymidine (Fig. 1).

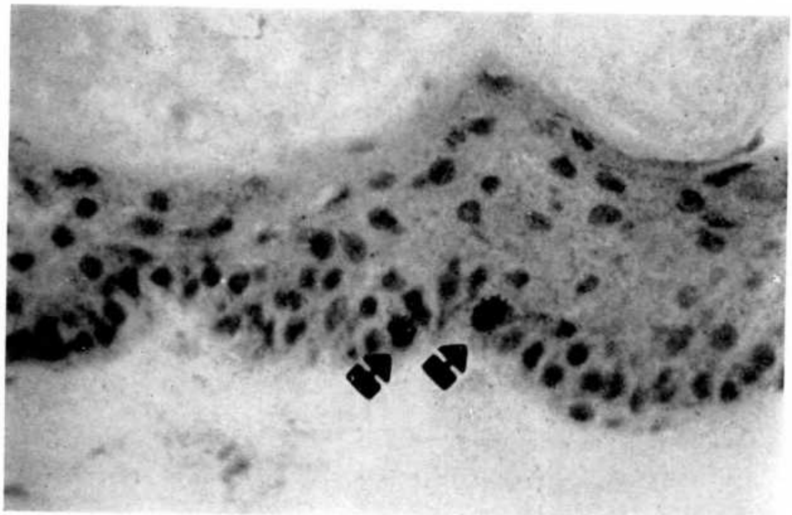

FIGURE 1. Autoradiograph of organ culture of human skin treated by cytochalasin B (2μg/ml) showing two labelled cells in basal layer indicated by arrows (cryostat section, H & E, ×90).

TABLE 1. The effect of cytochalasin B on epidermal cell migration *in vitro*. The figures refer to the number of specimens

	Concentration of cytochalasin B (μg/ml)					
Epidermal migration	Control	2×10^{-5}	2×10^{-3}	2×10^{-1}	1	2
0	0	0	0	2	5	7
+	4	1	2	2	2	0
++	5	1	0	4	0	0
+++	2	0	0	1	0	0
Totals	11	2	2	9	7	7

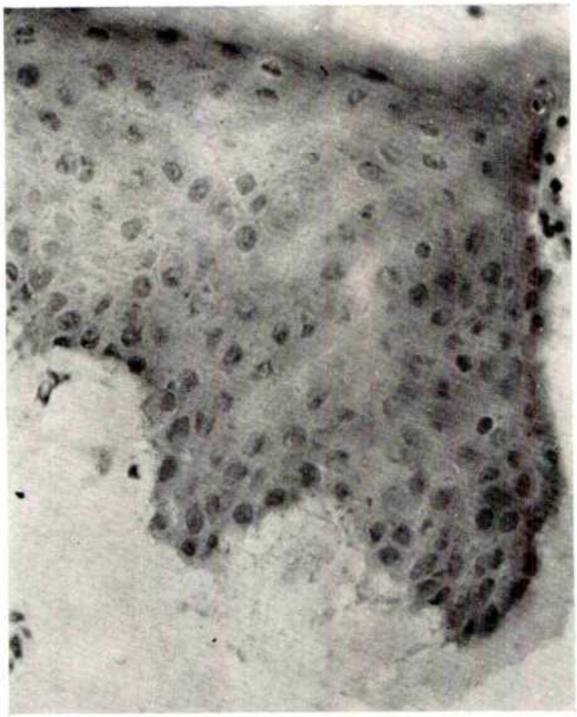

FIGURE 2. Edge of human skin explant showing 'heaped up' appearance (cryostat section, H & E, ×105).

TABLE 2. Labelling indices* for cultures treated with cytochalasin B and pulsed with tritiated thymidine in the last 4 h of a 48 h culture (±SEM)

Concentration of cytochalasin B	Labelling index	Numbers examined	P
0	1.2 ± 0.4	5	
1 μg/ml	1.6 ± 1.1	4	>5%
2 μg/ml	2.8 ± 1.5	5	>5%

* Number of labelled cells per 1000 basal cells, expressed as a percentage.

Effect of cytochalasin B

Set out in Table 1 are the results of culture of thirty-eight explants in the presence of concentrations of cytochalasin B ranging from 2×10^{-5} μg/ml to 2 μg/ml. It can be seen that cytochalasin at a concentration of 2 μg/ml always inhibited epidermal migration and in a concentration of 1 μg/ml epiboly was usually, but not always, inhibited. In concentrations of less than 1 μg/ml epidermal migration proceed normally. In some of the explants exposed to cytochalasin B in a concentration of 2 μg/ml there appeared to be a 'heaping up' or thickening of the epidermis at the edges (Fig. 2). Nuclear enucleation, such as has been seen in various cell types by Poste (1972) was not seen.

The number of epidermal cells incorporating tritiated thymidine in the cytochalasin treated specimens did not differ statistically from the controls (Table 2).

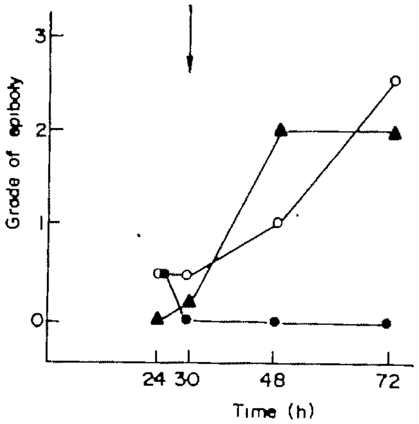

FIGURE 3. The effect of cytochalasin B on epidermal cell migration in organ culture. ○, without cytochalasin; ●, with cytochalasin B; ▲, cytochalasin B plus media change (arrowed) at 30h.

Reversibility of the effect of cytochalasin B

Thirty-six explants were used in these experiments to test the reversibility of the effects of cytochalasin B. Explants cultured initially in the presence of cytochalasin B (1 or 2 μg/ml) showed no significant epidermal cell migration, although the controls invariably did so. After culturing some explants in the presence of cytochalasin B (for 30 h) these were transferred to cytochalasin free medium. When examined after 18 and 42 h of additional culture these explants showed increasing degrees of epiboly. In Fig. 3 this is represented graphically for one experiment.

COMMENT

From the above experiments it may be concluded that cytochalasin B is inhibitory to epidermal cell migration *in vitro* and that this effect is reversible.

We have shown previously that epidermal cell movement is not accompanied by epidermal cell proliferation *in vitro* (Marks & Nishikawa, 1973), and this *in vitro* process of cell migration appears to be an energy dependent phenomenon (Gibbins, 1972, Marks *et al.*, 1972). However, there is little information concerning the cytological changes that take place in migrating keratinocytes.

Many cell types that have been investigated possess a system of cytoplasmic microfilaments (5–8 nm in diameter) which appear to be intimately involved in cell movement and change in cell shape (Wessels *et al.*, 1971). These microfilaments have, for example, been found in the microprocesses of various motile cells (Goldman & Follett, 1969) and Spooner & Wessels (1970) have demonstrated the importance of microfilaments in the change of shape that accompanies salivary gland morphogenesis.

It has been claimed that the microfilaments are disorganized in a reversible manner by the cytochalasins—a group of compounds of complex macrolide structure which are derived from fungi (Wessels *et al.*, 1971). Cytochalasin B has for example been shown to prevent the fusion of the neural tube (Linville & Shepard, 1972), to prevent axonal elongation *in vitro* (Yamada, Spooner & Wessels, 1970) and to inhibit the movement of melanin granules in melanocytes (Malawista, 1971). These reversible 'anti-movement' effects are not due to non-specific tissue damage caused by cytochalasin B and indeed, it has been claimed that the specificity of the action of these compounds makes them useful biological tools. Thus, it has been suggested that the reversible inhibition of a process involving movement by the cytochalasins implies the involvement of the cytoplasmic microfilaments. However, Goldman (1972) found that some cell types did not respond by becoming amotile and rounding up and this author has expressed reservation interpreting the actions of cytochalasin B on the microfilament system. Recently Carter (1972) has also cautioned against the assumption that the cytochalasins exert their action on the microfilament system.

Wikswo & Szabo (1972) have investigated the effect of cytochalasin B on melanocyte and keratinocyte cell cultures. Their ultrastructural observations indicated that the cytochalasin produced a marked effect on cell morphology and stopped the 'ruffling' of the cell membrane. They also observed marked changes in microfilaments and concluded that they thought that their results indicated that there is a correlation between the integrity of the microfilament system and the morphology and movement of pigment cells and keratinocytes. Although cytochalasin B stopped 'pigment donation' the contacts between keratinocytes and melanocyte were not disrupted. Our findings using cytochalasin B resemble those of other workers using other tissues, i.e. movements are reversibly inhibited, but give no clue as to the mechanism involved. Clearly cytochalasin B has a profound effect on the epidermis and further investigation of this aspect of its action is required.

ACKNOWLEDGMENT

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Effect of L-dopa on the seborrhoea of Parkinsonism

J.L.BURTON, MARGARET CARTLIDGE AND SAM SHUSTER

Wellcome Laboratories for Research into Skin Disease,
University Department of Dermatology, Newcastle-upon-Tyne 1

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SUMMARY

By studying the sebum excretion rate in patients with Parkinsonism before and during treatment with L-dopa we have shown that L-dopa reduces sebum excretion if seborrhoea is initially present, but has no effect on normal sebum excretion rates. The seborrhoea of Parkinsonism may be due to increased production of melanocyte-stimulating hormone, and our finding may have therapeutic implications in Parkinsonism and breast cancer.

In a preliminary communication we reported that L-dopa reduced the seborrhoea of Parkinsonism (Burton & Shuster, 1970). This was confirmed by Harville & Appenzeller (1971) but Cotterill *et al.* (1971), using an identical method to our own, found L-dopa had no effect on the sebum excretion rate (SER) in Parkinsonism. We have now investigated this discrepancy.

PATIENTS AND METHODS

Seven patients with idiopathic Parkinsonism were studied before and during treatment with L-dopa. The SER from forehead skin was measured by the method of Strauss & Pochi (1961) as modified by Cunliffe & Shuster (1969), and this was repeated when the patients had been taking maximal tolerable doses of L-dopa (2–5 g/day) for at least 6 weeks. All the patients were also taking oral anticholinergic drugs before and during treatment with L-dopa.

The results were compared with the SERs in a control group of thirty-seven male and forty-eight female subjects aged 40–80 years.

RESULTS

The SERs in the Parkinsonism patients in the present study are shown in Table 1. The mean SERs before and during L-dopa therapy did not differ significantly, but when the change in each individual patient was expressed as a percentage of the initial SER a small decrease became apparent. The mean percentage reduction in SER was 15·6 ± SE 6·4% after L-dopa and this was just significant ($P < 0·05$).

It seemed likely that the effect of L-dopa depended on the initial SER and to test this we pooled the results from our previous five patients and our present seven patients with those from the seven patients of Cotterill *et al.* (1971). This seemed permissible since exactly the same method for measuring SER was used in each of these three studies. It can be seen from Fig. 1 that the decrease in SER in individual patients is indeed a function of the initial SER. The same point is also apparent from a comparison with normal individuals. The normal SER varies with age and sex (Fig. 2) but from this data we have arbitrarily defined seborrhoea as 1·4 μg/cm^2/min or more in the males and 0·7 μg/cm^2/min or more in the females. Figure 3 shows the response to L-dopa in the two groups with and without an initial seborrhoea. In the eleven patients with initial seborrhoea the mean reduction in SER was 0·28 ± SE 0·05 μg/cm^2/min and this decrease was highly significant ($P<0{\cdot}001$). In the eight patients with a normal initial SER there was no significant reduction in SER during L-dopa therapy (mean 0·02 ± 0·06 μg/cm^2/min, $P>0{\cdot}05$).

TABLE 1. Effect of L-dopa on sebum excretion in Parkinsonism

Patient	Age	Sex	Sebum excretion rate (μg/cm^2/min) Before L-dopa therapy	Sebum excretion rate (μg/cm^2/min) During L-dopa therapy	Percentage change in SER after L-dopa
1	58	M	1·63	1·53	− 6
2	62	M	1·74	1·47	−14
3	47	M	1·59	1·08	−32
4	47	M	0·83	0·86	+ 4
5	57	F	0·55	0·30	−45
6	56	F	0·89	0·81	− 9
7	62	F	0·41	0·38	− 7
Mean ± SE			1·09 ± 0·21	0·92 ± 0·18	−16 ± 6

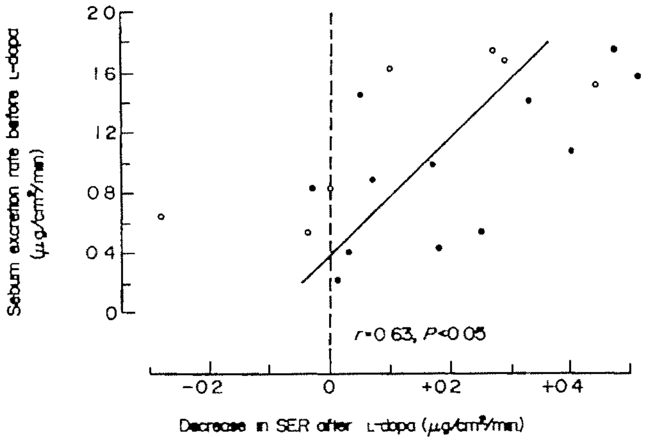

FIGURE 1. Effect of L-dopa on the sebum excretion rate in Parkinsonism. The data from the present study is pooled with the previous data of Burton & Shuster (1970) and Cotterill *et al.* (1971). ●, Females; ○, Males.

DISCUSSION

The present study has confirmed our finding that L-dopa decreases the seborrhoea of Parkinsonism

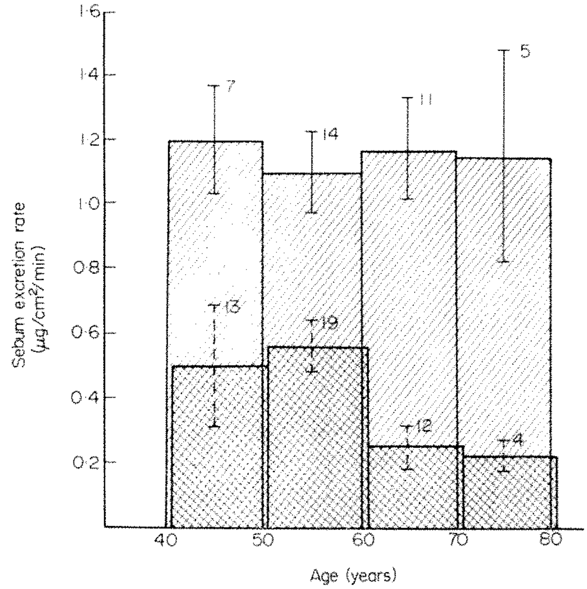

FIGURE 2. The normal sebum excretion rates in thirty-seven male (diagonal hatching, mean ± SE), and forty-eight female (diagonal cross-hatching, mean ± SE) control subjects of the same age range as the patients with Parkinsonism.

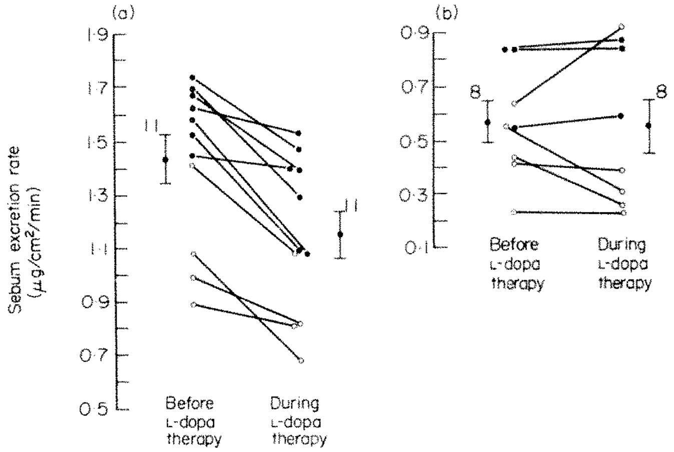

FIGURE 3. The response of the sebum excretion rate to L-dopa in Parkinsonism patients with (a) and without (b) an initial seborrhoea. ●, Males; ○, Females, mean ± SE.

(Burton & Shuster, 1970). Not all patients with Parkinsonism have seborrhoea and it now appears that L-dopa has no effect on the SER unless seborrhoea is present initially. This probably accounts for the discrepancy between our results and those of Cotterill *et al.* (1971), since eight of the twelve patients we studied had seborrhoea compared with only three of their seven patients and the SER decreased in all these three.

We have also shown that L-dopa has no effect on the SER of normal rats (Thody & Shuster, unpublished) or human subjects with severe acne (Burton *et al.*, 1971). It appears therefore that L-dopa decreases sebum excretion only where there is a specific abnormality of sebaceous gland control. The evidence suggests that the seborrhoea of Parkinsonism is induced by excessive secretion of a sebotrophic hormone (Burton & Shuster, 1970; Burton *et al.*, 1973). Since the neurological lesion of Parkinsonism leads to excess secretion of sebum and correction of the neurological deficit decreases the SER we conclude that the postulated sebotrophic hormone is under inhibitory control.

Prolactin and melanocyte-stimulating hormone (MSH) are the two pituitary hormones known to be under inhibitory control and we have already shown that prolactin is not sebotrophic in the hypophysectomized rat (Thody & Shuster, 1971) or the intact rat (Thody & Shuster, unpublished). Furthermore, removal of the neurointermediate lobe, the site of secretion of MSH in the rat, leads to a profound decrease in sebum secretion (Thody & Shuster, 1972). The general conclusion that the pituitary may control sebum production through the secretion of an MSH-like hormone might therefore explain our findings in Parkinsonism. The lesions of Parkinsonism presumably involve a centre concerned with control of the secretion of MSH-inhibiting factor (MIF); in Parkinsonism this centre is affected, less MIF is released and MSH secretion escapes from its normal inhibitory control and seborrhoea results. L-dopa restores the function of the affected centre, MIF is released normally and this inhibits MSH secretion which in turn decreases sebum production. This suggestion is supported by the finding that dopamine infusion in the rat can cause liberation of both hypothalamic inhibiting and releasing-factors (Kamberi, Mical & Porter, 1970a, b, 1971).

The absence of gross skin pigmentation in Parkinsonism does not rule out the action of an MSH-like hormone, since little is known of the physiological role of MSH and skin pigmentation may well prove to be its least important function. The administration of exogenous MSH is known to exacerbate Parkinsonism, presumably by decreasing mid-brain dopamine (Cotzias, Van Woert & Schiffer, 1967). This raises the intriguing new possibility that MIF–MSH control is by feedback of MSH on mid-brain dopamine.

An unexplained increase in sebum production also occurs in some patients with breast cancer (Krant *et al.*, 1968; Burton, Cunliffe & Shuster, 1970). The mammary glands and sebaceous glands have some similarities and it is not improbable that both the tumour and the sebaceous glands are responding to the same endocrine drive. There is evidence to favour the role of a pituitary trophic hormone other than gonadotrophin and adrenotrophin in the maintenance of breast cancer (Hayward *et al.*, 1970) and it has been suggested that L-dopa may produce clinical remission of breast cancer by inhibiting prolactin release (Stoll, 1972; Minton & Dickey, 1972). There seems to be an obvious parallel with Parkinsonism, and in view of the finding in the rat that MSH is strongly sebotrophic whereas prolactin is not, the alternative that MSH may provide the endocrine drive in breast cancer is now a distinct possibility.

A study of circulating MSH in patients with Parkinsonism and breast cancer would be worthwhile and so would a therapeutic trial of MIF.

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The response of hairless mice to staphylococcal epidermolytic toxin

J.P.ARBUTHNOTT, JANET KENT AND W.C.NOBLE

Department of Microbiology, University of Glasgow and Department of Bacteriology, St John's Hospital for Diseases of the Skin, Homerton Grove, London

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SUMMARY

A study of the effect of staphylococcal epidermolytic toxin in the progeny of Ahr × hrhr cross-bred mice revealed that hrhr mice were sensitive to the toxin at between 16 and 30 days of age. Such animals were more sensitive per g body weight than neonatal mice.

An extracellular protein from phage group II staphylococci capable of inducing epidermal splitting similiar to that seen in human toxic epidermal necrolysis (the scalded skin syndrome) has been purified in three laboratories (Kapral & Miller, 1971; Arbuthnott *et al.*, 1972; Melish, Glasgow & Turner, 1970, 1972). The bio-assay of this toxin, termed epidermolytic or exfoliative toxin, has involved subcutaneous injection of neonatal mice and monitoring for the appearance of a positive Nikolsky sign. It has been reported that mice more than 6 days old, the time when hair growth becomes apparent, are not susceptible to epidermolytic toxin (Arbuthnott *et al.*, 1971; Melish *et al.*, 1972). Indeed, Melish *et al.* (1972) state that 'adult animals appeared resistant to a single dose of as much as 25,000 exfoliating units'.

In the present study the response of hairless mice of different ages to epidermolytic toxin was investigated in an attempt to assess whether the susceptibility of mice to toxin was determined only by the age of the animals.

MATERIALS AND METHODS

Epidermolytic toxin

A freeze dried preparation of culture filtrate (Arbuthnott *et al.*, 1971) from a phage group II strain, 123A, of *Staphylococcus aureus* was used throughout. This was treated with the appropriate amount of α-antitoxin (Wellcome Research Laboratories, Beckenham, Kent) in order to neutralize staphylococcal α-toxin.

Mouse strains

Most experiments were carried out using the progeny obtained by crossing F_1 heterozygote, Ahr (haired) females and homozygote, hrhr (hairless) males; this yielded a progeny of which approximately 50% was hairless. In some experiments the progeny of AA × AA and hrhr × hrhr mice were used; only a small number of experiments could be carried out using pure line mice because of the small litters obtained.

Both hrhr (hairless) and Ahr (haired) mice exhibit hair growth at 6 days. However, hrhr animals begin to shed hair at approximately 16 days of age and by 21 days are hairless; hair loss begins at the nose and passes backwards towards the tail 3–5 days later. The hrhr progeny of the Ahr × hrhr cross could therefore be identified at 16 days of age.

Experimental procedure

Litters were randomized and the mice were weighed prior to injection. Epidermolytic toxin was administered subcutaneously on the basis of a unit dose per g body weight. Experimental animals were examined hourly for the appearance of a positive Nikolsky sign on light stroking of the skin.

RESULTS

Preliminary observations using pure line hrhr mice showed that staphylococcal epidermolytic toxin caused epidermal splitting in both 4-day and 23-day-old animals. A more detailed study of the response of mice of different ages was carried out with Ahr × hrhr cross-bred animals (Tables 1 and 2, Fig. 1a and b). In addition to establishing the susceptibility of adult hairless mice to epidermolytic toxin these findings show that mice aged between 5 and 10 days were less sensitive to epidermolytic toxin than those aged 1–3 or 18–26 days.

TABLE 1. Epidermal splitting in Ahr × hrhr mice injected subcutaneously with staphylococcal epidermolytic toxin*

Age (days)	Number of mice	Average weight of mice (g)	Number showing a positive Nikolsky sign at			
			1 h	2 h	3 h	4 h
1	9	1·45	0/9	9/9	9/9	9/9
3	10	2·3	0/10	9/10	10/10	10/10
5†	7	3·7	0/7	0/7	5/7	6/7
8†	10	3·8	0/10	0/10	1/10	5/10
10†	4	5·0	0/4	0/4	0/4	0/4
18‡	4	9·0	1/4	4/4	4/4	4/4
26‡	5	14·4	1/5	5/5	5/5	5/5

* Crude epidermolytic toxin was administered at a dose of 0·28 mg/g body weight.

† Some hair present in all animals at this age.

‡ These groups contained only hairless progeny.

A comparison of the susceptibility of neonatal (Ahr × hrhr) and adult hrhr (hairless) mice to varying doses of epidermolytic toxin is shown in Table 3; adult animals were found to be more sensitive than neonatal animals to toxin. Having shown the adult hairless mouse to be a suitable test animal for assaying staphylococcal epidermolytic toxin it was possible to establish that epidermolytic toxin was active when administered by the intraperitoneal route as well as by subcutaneous injection. Mice injected intravenously also showed epidermal splitting. However, since splitting began at the site of injection, at the tail, some direct cutaneous effect cannot be excluded.

Although the toxin is antigenic, giving rise to neutralizing and precipitating antibody in rabbits, it was

TABLE 2. Epidermal splitting in Ahr × hrhr mice injected subcutaneously with staphylococcal epidermolytic toxin*

Age (days)	Number of mice	Average weight of mice (g)	Number of mice showing a positive Nikolsky sign at	
			3 h	7 h
4	12	2·32	6/12	11/12
7–9†	13	5·5	1/13	1/13
12†	12	5·5	0/12	0/12
21‡	8	9·5	6/8	8/8

* Epidermolytic toxin was administered subcutaneously at a dose of 0·25 mg/g body weight.

† Hair present in all animals at this age.

‡ These groups contained only hairless progeny.

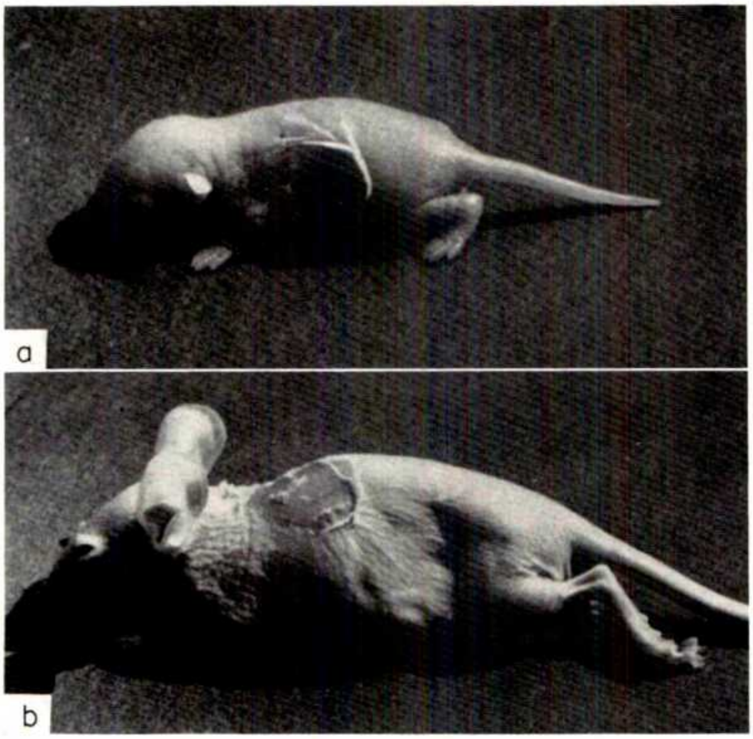

FIGURE 1. The effect of staphylococcal epidermolytic toxin (a) in a 3-day-old (Ahr × hrhr) mouse and (b) in a 26-day-old (hrhr) mouse. The animals were injected under the conditions described in Table 1.

TABLE 3. The susceptibility of neonatal and adult hairless mice to varying doses of epidermolytic toxin*

A 3-day-old mice†			B 26-day-old mice‡		
No. of mice	Dose of toxin (mg/g mouse)	No. showing positive Nikolsky sign at 3 h	No. of mice	Dose of toxin (mg/g mouse)	No. showing positive Nikolsky sign at 3 h
6	0·04	0/6	5	0·026	2/5
6	0·08	0/6	5	0·052	4/5
6	0·16	4/6	5	0·129	4/5
6	0·33	6/6	5	0·26	4/5
6	0·66	6/6	5	0·52	5/5
5	1·32	5/5	6	0·78	6/6

* Toxin administered subcutaneously.
† 3-day-old progeny of Ahr × hrhr cross.
‡ 26-day-old hrhr (hairless) mice.

noted that, of twenty hairless mice of differing ages which were challenged with a second dose of toxin 26 days after the first injection, seventeen give a positive Nikolsky sign in the second test.

DISCUSSION

A study of the response of the progeny of Ahr × hrhr cross-bred mice has shown that mice up to 5 days of age were sensitive to staphylococcal epidermolytic toxin and that mice became resistant to this factor at 8 days of age; animals remained resistant to toxin until at least 12 days of age. However, the hrhr progeny, which could be detected due to the shedding of hair, were sensitive to epidermolytic toxin at between 16 and 30 days of age. (No mice older than 30 days of age were tested). Although the appearance of resistance at 8 days is in agreement with earlier observations (Arbuthnott *et al.*, 1971; Melish *et al.*, 1972), the demonstration of epidermal splitting in hairless mice more than 16 days old indicates that the apparent resistance of haired animals of this age cannot be explained only in terms of the age of the mice.

Results of preliminary experiments using 12-day-old haired mice depilated with either Immac or Nair show that the skin of haired mice exposed by depilation is susceptible to staphylococcal epidermolytic toxin. However, these findings must be interpreted with caution, since treatment of the skin with depilating agents may, as a secondary effect, induce susceptibility to the toxin.

The use of neonatal mice in the bio-assay of epidermolytic toxin has presented certain problems. For instance (a) it is difficult to standardize the injection procedure in such small animals; (b) it is not possible to administer material intravenously in neonates, (c) such young animals have to be housed with a lactating female during the experiment and may suffer cannibalism (Arbuthnott *et al.*, 1971). For these reasons and because 3–4-week-old hairless mice are more sensitive to the action of staphylococcal epidermolytic toxin than are 3-day-old mice, we propose that, for some purposes, the former present a more suitable model for the bioassay of this toxin.

ACKNOWLEDGMENTS

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Skin sampling for *Candida* with adhesive tape

R.StC.BARNETSON AND L.J.R.MILNE

Department of Dermatology, Royal Infirmary and Mycology Unit, Western General Hospital, Edinburgh

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SUMMARY

A simple and convenient method for the collection of *Candida* is described using skin strippings with vinyl plastic adhesive tape. On comparison with the use of a wet swabbing technique, the yeast was cultured from significantly more specimens collected by the stripping method. It was also shown that this method may be useful in quantification of the growth of *Candida* on culture per unit area of skin.

Investigation of the carriage of the yeast *Candida* on the skin has been hampered by the lack of a really satisfactory method for its collection. The two most popular conventional methods have been scraping of the skin using a scalpel blade, and the use of wet swabs. Skin scraping is a good method only for sampling a scaly rash, but is uncomfortable for the patient and thus is not satisfactory for sampling normal skin nor for sampling several areas on the same patient. Wet swabbing, on the other hand, is easy to carry out and acceptable to the patient, but yields are difficult to quantify per unit area of skin.

A method of collection that has not been fully evaluated is the use of adhesive tape strippings. This is by no means a new technique in the collection of skin organisms. Porto (1953) described it in the diagnosis of *Tinea versicolor*, and Thomas (1960) used the method to assess the carriage of *Staphylococcus* quantitatively on the skin. More recently, Knudsen (1971) also found this technique useful in the collection and culture of both dermatophytes and yeasts.

The present study was therefore designed to compare the technique with a conventional method for sampling yeasts—in this case, wet swabbing.

MATERIALS AND METHODS

Scotch tape, number 681, a vinyl plastic tape marketed by the 3M Company, was selected on the basis of its water resistance, 'grab' and ability to remain flat on culture. Toxicity tests showed that the tape possessed no significant fungistatic properties. To facilitate handling, and to reduce contamination, tab ends were attached to the strips of 19 mm wide tape, these being made by folding Tagcraft Presson Labels (24 × 32 mm) over each end. The prepared tape was then applied to a microscope slide (Fig. 1).

The skin was sampled by detaching the tape and placing it over an area of skin, firming with the thumb to ensure close contact. The tape was then peeled off and replaced on the slide for transport to the laboratory. The same area of skin was also sampled using an ordinary cotton-wool swab, previously moistened with sterile distilled water. The order of sampling, which was randomized, was noted.

In the laboratory, the tape was reduced to a 40 mm length and placed with the adhesive surface in contact with the agar culture medium (malt peptone agar plus penicillin, streptomycin and actidione) (Fig. 2). Care was taken to remove any air trapped under the surface of the tape which was then peeled off the plate and repositioned permanently on a further adjacent area of the medium in the Petri dish. The area inoculated by the temporary siting of the tape was termed the 'replica' area. A Petri dish containing the same culture medium was inoculated with the wet swab. After incubation at 37°C for 48 h, the cultures were examined and the growth on both plates was recorded as profuse, moderate, scanty or absent. Where possible the number of colonies produced on the replica area was counted. All isolates were fully identified.

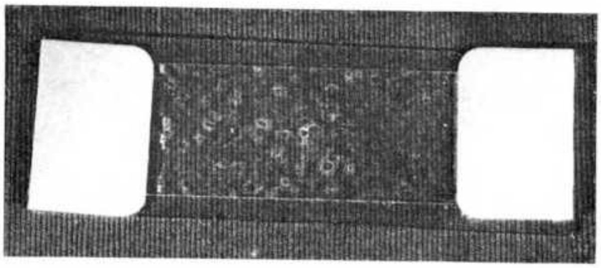

FIGURE 1. The prepared tape applied to the microscope slide for easy transportation.

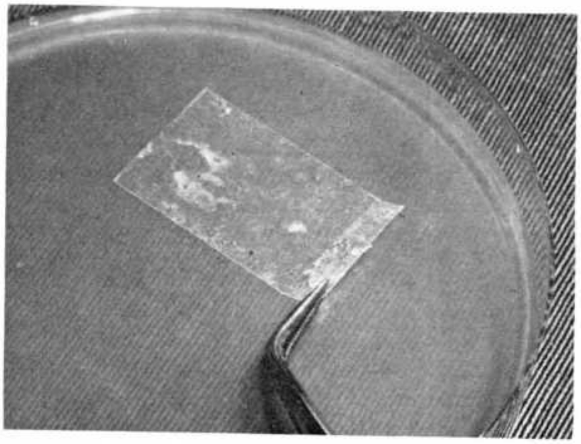

FIGURE 2. The tape reduced to a 40 mm length is placed with the adhesive surface in contact with the agar culture medium.

A smear was prepared from the wet swab after inoculation of the culture medium. In the case of the tape specimen, the microscope slide used for transport was also heat-fixed and Gram-stained. The presence of yeast cells and pseudo mycelial elements was recorded.

RESULTS

Initially 100 patients in a geriatric unit with essentially normal skin were sampled by skin stripping and wet swabbing of the right inguinal region. Seven of these patients were found to have *Candida* on the skin, the yeast being cultured by both types of collection from each. Five of these patients were then sampled from the following areas: groins, submammary folds, toe clefts, umbilicus and areas on the trunk. The results of the collections by both methods are shown in Table 1.

Patients in the Dermatology Unit with widespread rashes were also screened, and five were found to have *Candida* on the skin. As in the case of the geriatric patients, several areas were sampled similar to those listed above, from both normal and abnormal skin. The diagnosis of the underlying skin condition in these patients was: psoriasis (3), pemphigoid and acrodermatitis enteropathica. The results of these collections are also shown in Table 1.

TABLE 1. Comparison of positive cultures and positive microscopy obtained by the two methods*

	Culture		Microscopy	
	Swab	Strip	Swab	Strip
Geriatric patients				
1	4/9	6/9	3/9	3/9
2	2/8	3/8	1/8	0/8
3	0/8	2/8	0/8	0/8
4	4/8	4/8	0/8	2/8
5	3/7	5/7	0/7	0/7
Dermatology patients				
1	8/11	10/11	0/11	0/11
2	5/9	5/9	0/9	0/9
3	7/12	11/12	0/12	2/12
4	6/9	8/9	2/9	3/9
5	5/9	7/9	2/9	1/9
Total	44/90	61/90	8/90	11/90

* The figures denote the number of areas from which positive cultures or positive microscopy were obtained, per total number of areas sampled per patient.

DISCUSSION

As is seen in Table 1, if the results are totalled, *Candida* was cultured from more samples of skin by the stripping method (61/90), than by wet swabbing (44/90). Using the chi-squared test, this difference is statistically significant at the 2% level.

The number of samples which demonstrated yeast forms on microscopy proved to be much less (Table 1). There was no significant difference between either method of collection, but those samples showing yeast forms on microscopy gave a profuse growth on culture.

Quantification per unit surface of skin is also possible by the stripping method. In Fig. 3 culture plates from both methods may be compared. Thus, by this method, when growth of the yeast is profuse, numerous colonies are seen in the 'replica' area and these may be quantified per unit area of skin. Under the adhesive tape on the same culture plate, numerous colonies are also seen, but most have coalesced, making quantification impossible. A culture plate from the same area of skin obtained by wet swabbing is shown in comparison. Less profuse growths obtained by both methods are also

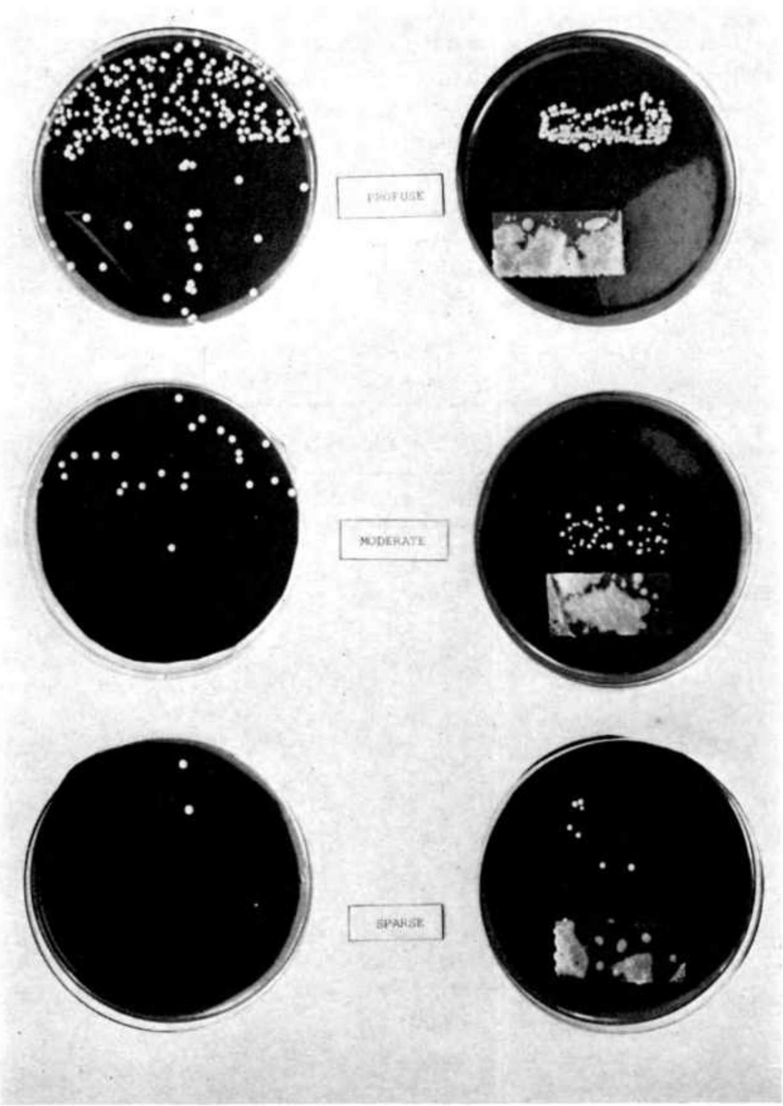

FIGURE 3. Culture plates from both methods of collection are compared. On the left are the plates prepared from wet swabs, and on the right the plates from similar areas obtained by skin stripping. The 'replica area' may be seen in the upper half of each plate from the stripping technique.

demonstrated in Fig. 3. When growth is very scanty from the stripping technique, no colonies are seen in the 'replica area', but colonies may be seen growing under the adhesive tape.

In conclusion it was found that skin stripping for the collection of *Candida* was easy and convenient. It was acceptable to the patients, and in this context we have also found it of particular use in collection of the organism from neonates.

ACKNOWLEDGMENTS

We have pleasure in acknowledging the advice of Professor P.J.Hare, Dr G.W.Beveridge and Dr J.A.Savin.

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Pharmacology and Treatment

Controlled trial of the treatment of atopic eczema with a urea–hydrocortisone preparation versus betamethasone 17-valerate

JOHN ALMEYDA* AND LIONEL FRY

Dermatology Departments of the London Hospital, London E.1, and St Mary's Hospital, London W.2

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SUMMARY

A double blind study has shown no statistical difference between the clinical response of fifty atopic eczema patients to topical treatment with 1% hydrocortisone in a stabilized 10% urea cream (Calmurid HC) and a 0·1% betamethasone 17-valerate cream (Betnovate).

Over the past decade in this country potent fluorinated steroid preparations, particularly betamethasone 17-valerate, have been widely used for the treatment of dermatological disease. Prolonged and intensive use of these preparations has been associated with unwanted effects such as striae formation (Epstein, Epstein & Epstein, 1963) adrenal suppression (Scoggins, 1962; Feiwel, James & Barnet, 1969), tinea incognito (Ive & Marks, 1968) and the aggravation and extension of rosacea (Sneddon, 1969; Weber, 1972).

Against this background there has been some movement towards encouraging a more restrained use of these substances, and to utilize less potent preparations where possible. Swanbeck (1968) showed that 10% urea cream increased the uptake of water by the horny layer or by scales of ichthyotic and psoriatic patients. As ichthyotic states are commonly found in atopic patients, he tried a 10% urea cream with 1% hydrocortisone added in twelve patients with atopic eczema, and noted that the clinical improvement was better than with 1% hydrocortisone alone. Therefore, in this trial a new preparation of 1% hydrocortisone in a stable carbamide (urea)-containing cream (Calmurid HC) has been compared with 0·1% betamethasone 17-valerate cream in the treatment of atopic eczema.

MATERIALS AND METHOD

Fifty patients with atopic eczema (most of whom had already received treatment) were examined, the extent and the severity of their atopic eczema was noted. They were each allocated two tubes of cream, one 10% urea and 1% hydrocortisone, and one 0·1% betamethasone 17-valerate, the identity of which were unknown to the clinicians. One treatment was applied twice daily for 2–3 weeks on one side and the second treatment to the other. Patients were randomized so that some treated their left sides with the urea and hydrocortisone and some their right sides. After treatment the patients were again examined and their progress categorized into excellent, good, none or deterioration, compared with the original state of the lesion.

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* Present address: Enfield Hospital Group.

In addition to their eczema, six patients had ichthyosis. A single patient with pityriasis lichenoides chronica was included in the study. The disease state was 'mild' in two patients, 'moderate' in thirty-two and 'severe' in sixteen. In twenty-six patients the nature of their previous topical steroid therapy was established.

Ages ranged from 9 months to 69 years.

RESULTS

(*a*) *All patients*

It was found that: in fifteen patients improvement was greater with betamethasone 17-valerate than urea hydrocortisone; in twelve patients improvement was greater with urea hydrocortisone than betamethasone 17-valerate; in twenty-three patients improvement was equally good with both preparations.

The responses obtained are summarized in Table 1.

TABLE 1.

	Excellent	Good	None	Deterioration
Urea hydrocortisone	13	25	11	1
Betamethasone 17-valerate	16	23	11	0

(*b*) *Children*

The results for patients aged 12 or less: in nine patients betamethasone 17-valerate gave the better response; in seven patients urea hydrocortisone gave the better response; in twelve patients the preparations gave equally good response.

The responses obtained are summarized in Table 2.

TABLE 2.

	Excellent	Good	None	Deterioration
Urea hydrocortisone	7	17	4	0
Betamethasone 17-valerate	9	15	4	0

(*c*) *Patients already treated with topical fluorinated steroids*

Of twenty-three patients who had previously been treated with a fluorinated steroid preparation: in eight patients urea hydrocortisone gave the better response; in six patients betamethasone 17-valerate gave the better response; in nine patients the preparation gave an equally good response.

These results are summarized in Table 3.

Statistical examination of the above data shows no significant difference between the effect of urea hydrocortisone and betamethasone valerate 0·1% cream in the treatment of atopic eczema. Six patients who had excoriated skin reported initial smarting when urea hydrocortisone was applied. The manufacturers in fact warn of this possibility because of the acid pH of the cream.

TABLE 3.

	Excellent	Good	None	Deterioration
Urea hydrocortisone	7	13	3	0
Betamethasone 17-valerate	8	9	6	0

DISCUSSION

The results show that in the treatment of atopic eczema a preparation of 1% hydrocortisone and 10% urea was as effective as a 0·1% betamethasone 17-valerate cream. In contrast to this result, a simple 1% hydrocortisone cream has been shown to be less effective in the treatment of atopic eczema than 0·1% betamethasone 17-valerate cream (Williams *et al.*, 1964).

It is believed that the poor effect of hydrocortisone in conditions characterized by hyperkeratosis, or located where the skin is naturally thickened, may be due to its poor penetration through the dry stratum corneum. Urea treatment is known to raise the water holding capacity of ichthyotic and psoriatic scales to that of normal skin *in vitro* (Swanbeck, 1968) and unpublished work shows that the treatment of ichthyotic patients with 10% urea cream raises the water holding capacity of their scales. This increase in water binding parallels clinical improvement (Grice, 1972). A similar improvement in the hyperkeratosis of eczema lesions may aid the penetration of the hydrocortisone, so producing an effect comparable with that of the readily penetrating fluorinated steroids, and in excess of that of an ordinary hydrocortisone cream.

In the group of patients who had previously had fluorinated steroid treatment for their eczema, there was a small trend in favour of urea hydrocortisone, compared with the patient group as a whole. Results in the patients below the age of 12 were not very different from those obtained from the adult group.

ACKNOWLEDGMENTS

We wish to thank Dr J.S.Pegum for referring patients, and to Pharmacia (G.B.) Ltd, for providing the urea–hydrocortisone (Calmurid HC) used in this study.

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Case Reports

Periodic shedding of the nails

ROBERT A.MAIN

Department of Dermatology, University of Aberdeen

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A girl aged 7 has been attending the skin clinic in the Children's Hospital of Aberdeen Royal Infirmary for some 4 years. She attended initially when she was 2 but when she was a few months old some of her toe nails and the left index finger nail had been seen to be deformed. The deformity became more noticeable and, at the age of 2, all the toe nails, except that of the left second toe, showed narrowing and loss of translucency and the great toe nails particularly were considerably thickened. The left index finger nail was also narrowed and showed increased convexity transversely (Fig. 1). Some of the nails had periodically loosened and fallen off, and this had continued to the present time. The great toe nails were particularly affected. Usually little or no inflammatory change occurred around the nail fold prior to the nail fall. Scrapings from the nails have shown no evidence of mycelium. Examination reveals no other evidence of skin disease. The hair is normal, and there are no changes in the oral mucous membranes. Her father, however, has epidermolysis bullosa, as also does the paternal grandfather and a paternal uncle and aunt. These members also have dystrophic nails not unlike the child's nails which loosen independently from time to time and are shed (Fig. 2).

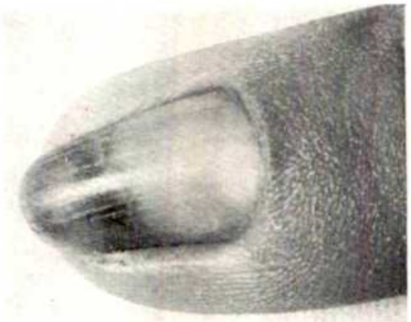

FIGURE 1

On re-examining three other families in this region with epidermolysis bullosa, who show only moderate blistering on the hands and feet, all the members showed involvement of the nails. A little boy aged 1½ had already shed two nails that have regrown, slightly discoloured, thicker and narrowed transversely. Another little boy aged 9 has lost six nails which have fallen frequently and, as they are replaced, on each occasion the nail again shows progressive diminution in size, increased thickening and loss of translucency. One father has no remaining finger nails while the other has only three apparently normal nails. In the third family, a woman aged 40 has finger nails which show similar narrowing and thickening with occasional loss, which she thinks occurs about twice a year.

It would seem from the appearance of this child's nails and from the family history of epidermolysis bullosa that she may well have epidermolysis bullosa affecting the nails only.

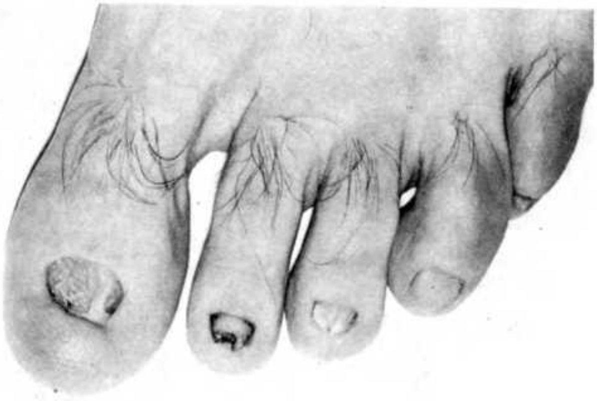

FIGURE 2

The deformity of the nails in this child is very similar to that described by Samman (1965) under the title 'Periodic Shedding'. It would seem that 'periodic shedding' of the nails may, in some instances, be an expression of epidermolysis bullosa.

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Allergic contact dermatitis from cedar wood (*Thuja plicata*)

E.BLEUMINK, J.C.MITCHELL AND J.P.NATER

Department of Dermatology, State University, Oostersingel 59, Groningen, The Netherlands, and Department of Medicine, Division of Dermatology, The University of British Columbia, Vancouver, Canada

Accepted for publication 26 October 1972

SUMMARY

A case of allergic contact dermatitis caused by the heart-wood of western red cedar (*Thuja plicata*) is reported. The workman, after exposure to woods for 2 years in a mill, developed an acute dermatitis of his face, hands and arms. Avoidance of contact with wood cleared the symptoms. Patch tests with extracts of nineteen different wood species revealed a strong reaction to western red cedar. Patch tests with the various components present in the wood showed positive reactions to gamma-thujaplicin, 7-hydroxy-isopropyltropolone and also to thymoquinone. Although thymoquinone was found to be a strong skin irritant, tests performed in two other patients with eczema indicated thymoquinone to be a potent allergenic component as well.

Wood of *Thuja* species is occasionally referred to as irritant, or as producing respiratory symptoms (Sachs, 1926; Touton 1925). Until recently, no comprehensive description of adverse reactions to red cedar wood had appeared in the literature. During the last two decades the use of western red cedar (*Thuja plicata*) in the timber industry has increased considerably. This may be the reason why several detailed reports on adverse reactions to this wood are now appearing (Chang-Yeung *et al.*, 1971; Gandevia & Milne, 1970; Komatsu, 1964; Mitchell, *et al.*, 1969; Sosman *et al.*, 1969). Several studies indicate that the fine sawdust from the heart-wood of western red cedar and other species belonging to the plant family Cupressaceae may lead to respiratory symptoms resembling asthma (Chang-Yeung *et al.*, 1971; Gandevia & Milne, 1970; Komatsu, 1964; Sosman et al., 1969). The symptoms have been attributed both to hypersensitivity reactions and to nonspecific toxic or irritant effects of inhaled materials (Gandevia & Milne, 1970; Komatsu, 1964; Sachs, 1926).

Another type of reaction is 'cedar poisoning' occurring in forest workers in Canada. Cedar poisoning is a misnomer for allergic contact dermatitis resulting from exposure to species of epiphytes present on the *barks* of the trees (Mitchell & Armitage, 1965; Mitchell *et al.*, 1969). *Frullania nisquallensis* (a leafy liverwort) is one of the most important causes of contact dermatitis among workers in the forests of British Columbia (Mitchell *et al.*, 1969); lichens have also been found to be allergenic (Mitchell & Armitage 1965). This type of reaction has to be distinguished from contact dermatitis occurring after contact with the *heart-wood* of red cedar. In the present communication, we report the first more detailed study of allergic dermatitis to red cedar wood and the identification of immunologically active components.

CASE REPORT

A man, aged 51 years, had worked for 2 years in a saw mill when, in July 1971, an itching vesicular and weeping dermatitis developed on his face, the dorsa of his hands and the flexures of his forearms. There was no previous personal or family history of eczema, asthma or hayfever. In-patient treatment for 2 weeks resulted in clearing of the dermatitis. A return to work in the same factory, however, caused within some days a relapse of the dermatitis. Patch tests revealed a strong delayed cutaneous reaction to extracts of wood from western red cedar and a weaker allergy to deal (*Picea*). During his work he regularly came into contact with the fine sawdust of these two woods. Avoidance of contact with wood cleared the symptoms.

PATCH TESTS

A set of screening patch tests was used according to the methods described by the International Contact Dermatitis Research Group (Malten, 1971). Substances tested were the allergens 0001–0020 recommended by the same group (Malten, 1971). In addition, patch tests were carried out with ethanol extracts of fine sawdust from nineteen different species of woods, which are regularly used in Western Europe (Table 1).

TABLE 1. Test series various woods*

Trade name	Botanical name	Plant family name
American walnut	*Juglans nigra* L.	Juglandaceae
Iroko	*Chlorophora*	Moraceae
Satiné rubané	*Brosimum paraense* Hub.	Moraceae
Coromandel	*Diospyros*	Ebenaceae
(Grand) Bassam mahogany	*Khaya ivorensis* A. Chev.	Meliaceae
(American) ash-wood	*Fraxinus*	Oleaceae
Teak	*Tectona grandis* L.J.	Verbenaceae
(European) beech	*Terminalia superba* Engl. et Diels	Combretaceae
Afzelia	*Afzelia*	Leguminosae/ Caesalpiniaceae
California redwood	*Sequoia sempervirens* Endl.	Taxodiaceae
Deal	*Picea abies* Karst	Pinaceae
Azobé	*Lophira alata* Banks	Ochnaceae
Sipo	*Entandophragma utile* Liebl.	Meliaceae
(European) Oak	*Quercus robur* L.	Fagaceae
Panga panga	*Milettia stuhlmanii* Taub.	Leguminosae/ Papillionaceae
Western red cedar	*Thuja plicata* D. Don.	Cupressaceae
Parana pine	*Araucaria angustifolia* O. Ktze.	Araucariaceae
Abura	*Mitragyna*	Rubiaceae
Pitch pine	*Pinus celliotti* Engelm.	Pinaceae

*A selection of woods used in the Netherlands. Other important woods, not included in the test series, are for example rose wood, afromosia, wengé, Oregon pine and European walnut.

The extracts were prepared in the following way: 15–20 g of fine sawdust was shaken for 2 h with 200 ml of 96% ethanol (at 20°C). This treatment was repeated once. The combined extracts were concentrated in a vacuum rotatory evaporator to about 20 ml and stored at 4°C.

For patch testing, the extracts were diluted to a 10% concentration. Aqueous and acetone extracts were also prepared in a similar way from western red cedar and deal. Furthermore, tests were performed with pure chemicals present in the wood of *Thuja* species (Barton & MacDonald 1971): gamma-thujaplicin, beta-thujaplican, thujic acid, methyl-thujate and 7-hydroxy-4-isopropyltropolone were tested as 1% solutions in ethanol. These substances were obtained from Dr G. M. Barton, The Western Forest Products Laboratory, Vancouver B.C. Carvacrol (technical, lot. nr 711-1), thymoquinone (lot. nr 1–4), benzohydroquinone, methyl-*p*-benzoquinone (lot. nr 711–3A), methylhydroquinone (lot. nr 713–1) and thymol (lot. nr 711–1) were purchased from Eastman–Kodak (Rochester N.Y.). The latter five components have not been found in red cedar wood (Barton & MacDonald, 1971), but were tested because their chemical structure is closely related to carvacrol and because some of these components are present in other 'cedar' species (Calnan, 1972). Tests were made with 1% solutions in ethanol.

On the patches 0·010–0·015 ml of these solutions (=0·10–0·15 mg material) were applied. The above substances were also tested in control persons.

RESULTS AND DISCUSSION

The results of the patch tests are shown in Table 2. Of the nineteen wood species which were assayed for their skin reactivity, western red cedar gave three positive reactions, whilst deal induced only weak responses. The allergenically active components of western red cedar proved to be water and ethanol soluble. Tests in ten control subjects were all negative.

In addition, tests were done with pure chemicals present in the wood of western red cedar: beta-thujaplicin, gamma-thujaplicin, thujic acid, methylthujate and 7-hydroxy-4-isopropyltropolone. Of these, 7-hydroxy-4-isoproplytropolone and gamma-thujaplicin showed positive reactions in this patient. Tests in three control persons were negative. The two components, belonging to the group of the tropolones, are chemically closely related and may well cross-react (see Fig. 1). Beta-thujaplicin, differing from the gamma-component only in the position of the OH-group on the ring structure, did not show cross-reactivity in this instance.

It is interesting to note that tropolones, which form the major part of the volatile components of western red cedar, have fungicidal properties and can act as adrenergic beta-receptor blocking agents (Barton & MacDonald, 1971).

Furthermore, patch tests were carried out with 1% solutions of carvacrol (a phenolic component) and thymoquinone (a type of *p*-benzoquinone) and related chemicals not present in the woods tested, viz. thymol, methylhydroquinone and methyl-*p*-benzoquinone. Of these, thymoquinone and methyl-*p*-benzoquinone induced strong patch test responses. Additional testing in control subjects with 1% and 0·1% solutions of the latter five components showed, in four of six persons so tested, positive reactions to 1% solutions of the thymoquinone and methyl-*p*-benzoquinone; 0·1% solutions were found negative. Thymoquinone (2-isopropyl-5-methylbenzoquinone) occurs in oils of various wood species including Pinaceae and Cupressaceae and is known as a skin-irritating substance. This is also the case for methylbenzoquinone. Recently, Calnan (1972) described two patients who developed allergic contact dermatitis with incense cedar wood (*Libocedrus decurrens*) used in ordinary 'lead' pencils. Patch tests revealed strong responses to extracts of incense cedar and western red cedar and to 1% solutions of thymoquinone and hydrothymoquinone.

Tests in controls were not reported and irritancy can therefore not be excluded. However, Calnan's

TABLE 2. Patch tests with wood extracts and chemical components

	48 h	72 h	96 h	168 h
Routine patch tests				
Allergens 0001–0020	–	–	–	NR*
Wood series (Table 1, 1–19)				
Western red cedar	3+	3+	3+	NR
Deal†	–	–	+	NR
Western red cedar:				
Water extr. 10%	2+	3+	NR	NR
Ethanol extr. 10%	2+	3+	NR	NR
Acetone extr. 10%	–	–	NR	NR
Deal:				
Water extr. 10%	2+	–	NR	NR
Ethanol extr. 10%	–	–	NR	NR
Acetone extr. 10%	2+	2+	NR	NR
Frullania nisquallensis	±	–	NR	NR
Gamma-thujaplicin	2+	2+	NR	NR
Beta-thujaplicin	–	–	NR	–
Thujic-acid	–	–	NR	–
Methylthujate	–	–	NR	–
7-hydroxy-isopropyltropolone	–	2+	NR	+
Carvacrol	–	–	–	NR
Thymoquinone	3+	3+	3+	NR
Methyl-hydroquinone	±	±	±	NR
Methyl-*p*-benzoquinone	3+	3+	3+	NR
Thymol	–	–	–	NR

*NR, reaction not read.

†Other wood extracts were negative.

(1972) conclusion that thymoquinone may act as a allergenic component is supported by additional findings obtained by us in two patients. The relevant data of these patients are recorded in Table 3. As may be observed from this table, 0·1% solutions of thymoquinone (which were not irritant in control subjects) give rise to strong positive reactions in both patients. Carvacrol, also occurring in various wood species, gave a positive reaction in one patient. The components chemically related to thymoquinone (methylquinone and methylhydroquinone) were also found to be positive.

It is noteworthy that patch tests with primin (the active allergen of *Primula obconica*), which is also a quinone derivative, proved to be negative in these patients, indicating that primin and thymoquinone do not cross-react.

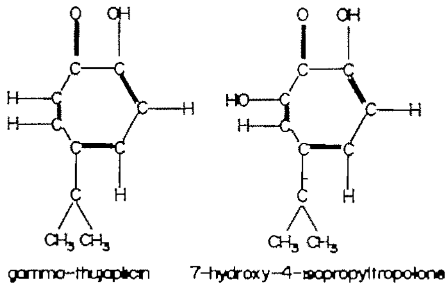

FIGURE 1. Ring structures of gamma-thujaplicin and 7-hydroxy-4-isopropyltropolone.

TABLE 3. Patch tests with phenolic and quinone components

	I	II
Clinical symptoms	Acute dermatitis of the face	Hand eczema
Routine patch tests	–	Wood tar 2+
Wood series 1 t/m 19	All extracts 2+	–
Carvacrol:		
1%	2+	–
0·1%	2+	–
Thymoquinone:		
1%	4+	4+
0·1%	4+	4+
Methyl-hydro-quinone:		
1%	2+	2+
0·1%	2+	–
Methyl-*p*-benzoquinone:		
1%	4+	3+
0·1%	2+	2+
Thymol:		
1%	–	–
0·1%	–	–
Primin 1%	–	–

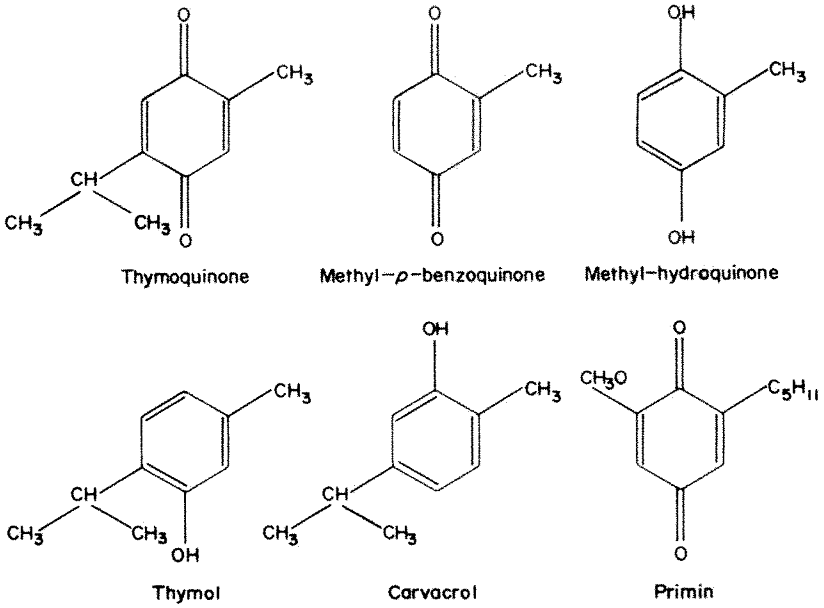

FIGURE 2. Ring structures of carvacrol and related chemicals.

Therefore, the nature of the (alkyl) substituents seems to be of great influence on the allergenicity of the quinone components. A similar conclusion may be drawn from the results obtained in the study of Hjorth, Fregert & Schildknecht (1969) on primula allergy.

Our findings indicate that western red cedar contains at least three active allergens viz.: gamma-thujaplicin, 7-hydroxy-4-isopropyltropolone and carvacrol. Although thymoquinone has not been found in western red cedar wood (Barton & MacDonald 1971) the particular patient showed strong skin reactions to it.

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Allergic contact sensitivity to gold with unusual features

HOOSSIK PETROS AND A.L.MACMILLAN

Department of Dermatology, Addenbrooke's Hospital, Cambridge

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SUMMARY

A case of allergic contact sensitivity to gold is reported. In addition to the usual eczematous manifestations of allergic contact dermatitis, the patient developed a striking bilateral papular (dermal) reaction to gold ear-rings. We suggest that both types of lesion are manifestations of allergic contact sensitivity to gold.

Reactions develop in about 30% of patients who receive gold systemically (Hartfall, Garland & Goldie, 1937) and the majority involve the skin. But allergic contact sensitivity to gold is exceedingly rare. Recently, Elgart & Higdon (1971) reviewed seven cases reported in the English literature and added their own case, where a gold dental appliance was involved. The same patient was presented at the Atlantic Dermatological Conference (Elgart, 1972). We may add two other published reports: one by Bowyer (1967), in which he comments on epidermal and prolonged dermal reactions to patch testing with gold salts, and the other by Cowan (1960).

The following case illustrates an allergic contact sensitivity to gold in a patient with some unusual and interesting features.

CASE REPORT

A housewife aged 29 attended with the complaint of 'lumps' and irritation of her earlobes. Thirteen months previously her earlobes were pierced and after 6 weeks the gold sleepers were removed; there were no immediate complications. Six months after the piercing, while wearing gold ear-rings, she started to develop itching, weeping and soreness of the earlobes, which was followed by 'swelling' around the puncture marks. She continued to wear the ear-rings for another 3–4 months and only stopped doing so 1 month before attending the out-patient clinic. In the meantime she was treated with different antibiotics without benefit.

On questioning she admitted that after the onset of earlobe symptoms she developed irritation and itching under her gold wedding ring. In addition she experienced similar symptoms, but more intensely, under her gold wrist watch. The latter symptoms had disappeared since she attached an adhesive tape to the back of the watch.

There was no personal or family history of atopy but she gave a history of irritation to metal clips and zip fasteners. She has never received gold therapeutically, nor has gold been used in her dental fillings.

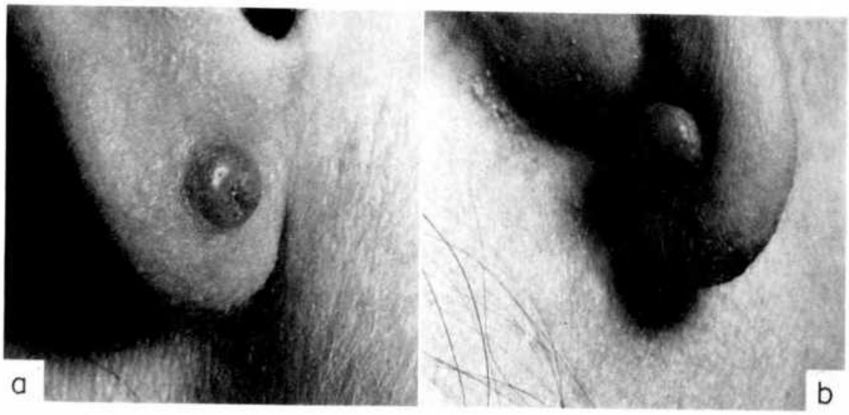

FIGURE 1. Earlobe lesions in the patient allergic to gold, (a) lateral and (b) medial views of the right earlobe. At the centre of each papule there is a depression corresponding to the site of ear piercing.

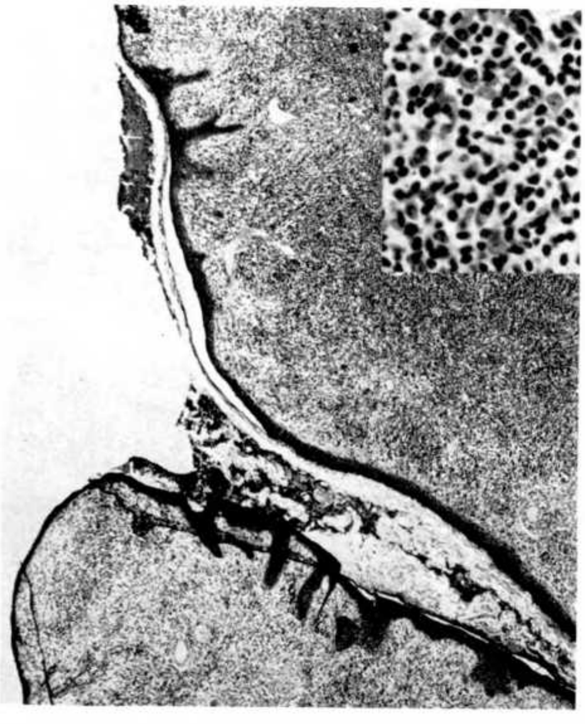

FIGURE 2. Photomicrograph of an excised earlobe papule showing the extreme density of the dermal round cell infiltrate and the central canal due to ear piercing. The folding of the epidermis at the lower edge of this canal is an artefact (H & E, × 25). Insert shows an area of the infiltrate of lymphocytes and plasma cells at higher magnification (× 280).

On examination there were flesh-coloured, large, prominent papules, without eczematous changes, around the medial and lateral puncture marks on both earlobes (Fig. 1). Eczematous changes were present under the wedding ring.

In addition to the routine battery she was patch tested with 0·5% aqueous gold chloride. The allergens were placed on A1-test (Imeco, Astra Agency, Stockholm) and fixed with Paragon (T.J. Smith & Nephew Ltd, Hull, England) tape. The results were read at 72 h. She gave positive results to the gold chloride (+ + +), 2·5% nickel sulphate (+ + +) and 1% cobalt chloride (+ +). The patient did not consent to an intradermal test.

A biopsy from one of the ear lesions showed a very dense infiltration of the dermis with lymphocytes and plasma cells. There were no significant changes in the epidermis (Fig. 2).

The earlobe lesions were slow in their response to intralesional injections of triamcinolone and the patient found the injections very painful. Since she was anxious to get rid of them on cosmetic grounds, the residual lesions were excised.

The patient was instructed to avoid all gold jewellery, and when we saw her again in 3 and 6 months time she was completely symptom-free and wore a silver wedding ring with impunity.

DISCUSSION

There seems to be general agreement that the stability and relative insolubility of gold accounts for the paucity of allergic contact sensitivity to this precious metal. Kligman (1966) has demonstrated the strong sensitizing potential of gold chloride under the conditions of his 'maximization' test. He believes that gold would be as troublesome as nickel if it were as readily solubilized by skin secretion. Wells (1965) has shown the release of nickel from metal objects when they were placed in a solution resembling sweat. We know of no comparable studies in relation to gold. But the amount of gold that actually dissolves in sweat must be infinitesimal (Fox, Kennedy & Rostenberg, 1961). However, in the reported cases quite a number of gold sensitive patients reacted more readily to the offending jewellery and found their symptoms more troublesome in warm weather. Another aspect of allergic contact reactions to gold, that has been commented upon by different authors, is the persistence of reactions for weeks and even months after contact with the offending agent has ceased (Shelley & Epstein, 1963; Bowyer, 1967; Comaish, 1969).

Of the nine previously reported cases of contact gold sensitivity, six involved gold jewellery. Two of these showed signs of eczematous contact dermatitis on wearing gold ear-rings (Cowan 1960; Elgart & Higdon, 1971). In one case gold sensitivity was initiated by ear piercing. In our patient too, the gold sensitivity followed the ear piercing and wearing of gold ear-rings.

Although we did not see our patient initially, on the basis of the history it seems likely that in the beginning she developed an eczematous contact reaction of the earlobes, but what makes this case unusual and striking is the subsequent development of the persistent papular lesions on both earlobes. Papular eruption due to contact sensitivity to gold has been reported (Shelley & Epstein, 1963). Their patient developed a chronic papular rash after he had rested his bare forearm on crystals of gold trichloride. A biopsy taken from the patient showed dense infiltration of the dermis with lymphocytes and plasma cells, similar to the findings in our case. They believe that in their case the allergen was a strong primary irritant which damaged the epidermis and entered the dermis where it later called forth a prolonged lymphoid response of lymphocytes and plasma cells. The authors consider this an example of non-eczematous (dermal) response to allergic contact sensitivity to gold. They emphasize that this unusual dermal response is based on the same delayed hypersensitivity immune mechanism which is responsible for the more common eczematous allergic contact sensitivity. Our patient, in addition to the usual manifestations of eczematous allergic contact sensitivity, presented a marked papular (dermal) reaction in the earlobes. It is suggested that the ear piercing and wearing of the ear-rings long after the onset of allergic symptoms, created favourable conditions for intimate contact with the allergen and a dermal locale for prolonged antigen–antibody cell reaction.

ACKNOWLEDGMENT

We would like to thank Dr R.H.Champion for permission to publish the details of this patient.

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A case of systemic multinodular mastocytosis

LJ.STANOEVA AND D.KONSTANTINOV

Department of Dermatovenereology, University of Skopje, Yugoslavia

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SUMMARY

A case of systemic multinodular mastocytosis in a girl aged 6 months is described.

The disease started at 3 months with numerous nodules over the entire body, including palms and soles. Severe itching and dermographism were also present. Transient bullous lesions appeared on the scalp, chest and limbs.

Enlargement of the liver, and of lymph nodes in both inguinal regions, was found. On X-ray examination of the skeleton osteoporotic and osteosclerotic changes were observed. X-ray examination of the gastro-intestinal tract showed nodules of the jejunal mucosa. Yellowish punctiform lesions were present in the fundus oculi.

CASE REPORT

I.M., a girl aged 6 months with no family history of similar disease: no consanguinity of the parents.

The present condition started 3 months after delivery, when the child's parents noticed nodules on the chest, arms and shins, accompanied by severe itching. The lesions spread in a very short time to other parts of the body including face, palms and soles. Sometimes bullae appeared on the scalp, neck and chest.

On examining the child, the only positive findings were an enlarged liver, about 3 cm below the costal margin, and enlarged lymph nodes in both inguinal regions. The child was small for her age. Dermatological state: over the entire body including scalp, face, palms and soles were numerous yellowish nodules in and below the skin, 0·5–1·0 cm in diameter. Fundus oculi: there were present numerous yellowish punctiform lesions which resembled the skin lesions. Laboratory findings: blood count RBC 2,670,000, WBC 8900, eosinophils 1%, neutrophils 31%, lymphocytes 65%, monocytes 1%. Urine normal. Liver function tests normal apart from slight increase in SGOT activity to 50 units/ml (normal 20–40) and SGPT 76 units/ml. Serum proteins and electrolytes normal. Cholesterol 120 mg/100 ml. Coagulation factors: prolonged bleeding time, platelets 150,000. Myelogram: reduced number of red cells and hyperplasia of leukocytes. Groups of lymphoid elements with abnormal structure were seen. Typical mast cells were not seen in the bone marrow. On X-ray examination of the long bones and hands osteosclerotic and osteoporotic changes were seen. X-ray examination of the gastro-intestinal tract showed in the proximal segments of the jejunum numerous mucosal nodules 3–4 mm in diameter. Some nodules were also present in the ileum.

Course and treatment of the disease

The child was hospitalized on two occasions (1969–70). During the first admission she was treated

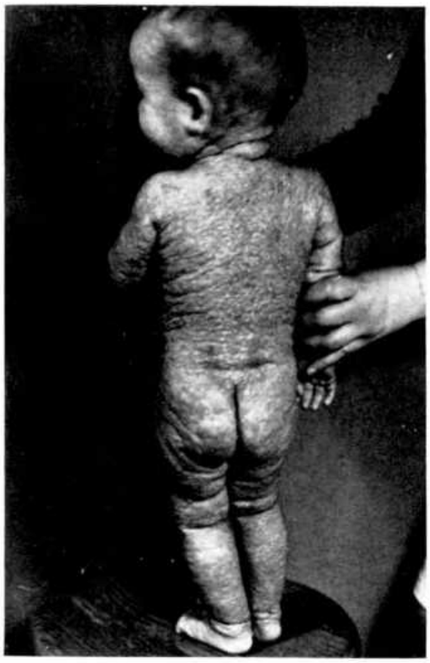

FIGURE 1. Generalized cutaneous nodules.

with reserpine (0·3 mg daily) for 7 days and after 1 month the treatment was repeated. Between the courses of reserpine, corticosteroids were given (15 mg daily). Local changes were treated with corticosteroid ointments. Antihistamines were also given for a prolonged time. Pruritus and dermographism improved and the child's condition was very good after 2 months when she was discharged. The nodules had greatly improved and in some regions (face and arms) they had actually disappeared.

During the second hospitalization, 6 months later, she was given one course of reserpine with corticosteroids. The skin lesions resolved satisfactorily. Histopathologically, the skin changes after

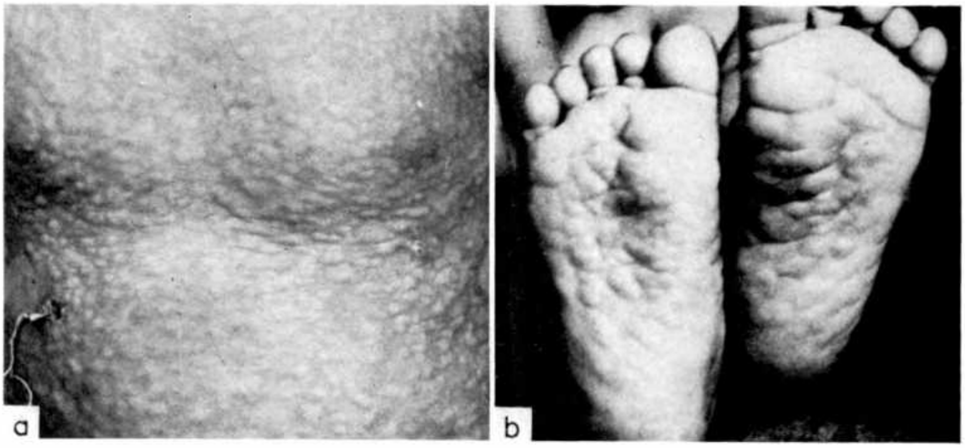

FIGURE 2. Nodules on the trunk (a) and soles (b).

reserpine therapy clearly showed disintegration of the metachromatic granule of the mast cells, many of them lying outside the cells.

Histological findings: nodular accumulations of mast cells were present in the mid-dermis. The infiltrate is composed predominantly of spheroidal cells. Liver biopsy showed mast cell infiltration of the sinusoids.

DISCUSSION

The diagnosis of xanthelasmoid multinodular mastocytosis was proved histologically. Systemic involvement was established by clinical, biochemical, and X-ray examinations. Urtication on friction was also present in our case, even after the nodules subsided. The sign of Popof (1957) (bulla formation on CO_2 pressure) was always positive. In the bulla contents there were mast cells. Spontaneous blistering was often seen on the neck and chest.

Pruritus at first was so intense that the child's health was impaired. The disease appeared within the first 3 months of life. According to Randazzo (1954), Udovikoff (1969) and others, the onset of mastocytosis is usually within 6 months after delivery. The association of bone lesions with cutaneous changes of mastocytosis is frequent according to Sagher, Cohen & Schoor (1952) and Sagher & Schoor (1956). Pulmonary lesions have rarely been described (Graciansky *et al.*, 1954). Barker (1957) found reticulate pulmonary changes radiologically and Lennert (1962) was able to demonstrate pulmonary mast cell infiltration in four cases. The lymphocytosis (65%) in our case deserves comment. Tolman (1935) found lymphocytosis (even over 80%) in a number of cases of systemic mastocytosis. Impaired coagulation in our patient caused prolonged bleeding after skin biopsy, as in the case described by Villanova, Gastillo & Pinol (1961). We were able to prove on X-ray examination the presence of infiltration of the intestinal wall, but the mastocytic nature of the infiltrate was not proved.

Hepatomegaly was present in our case but function tests were almost normal; only transaminase activity was slightly increased. Liver biopsy showed pathological changes with mast cells in the sinusoids. Hepatosplenomegaly is often found in systemic mastocytoses according to Asboe-Hansen & Kaalund-Jørgensen (1956).

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Comment

XERODERMA PIGMENTOSUM

Xeroderma pigmentosum (XP) is characterized by sensitivity of the skin to ultraviolet light (UVL) which eventually leads to the formation of malignant neoplasms. In 1925, Siemens & Kohn showed that it is genetically determined, with an autosomal recessive pattern of inheritance.

The effects of UVL at the molecular level have been studied in microorganisms in which UVL produces abnormal cyclobutyl-pyrimidine dimers in the DNA. These 'lesions' are repaired in the dark by excision of the dimers, and repair replication and rejoining of DNA strands. Similar experiments with fibroblasts from patients with XP revealed that the repair replication occurs at a reduced rate or not at all, and that the amount of unscheduled synthesis is negligible when compared with normal fibroblasts (Cleaver, 1968). These findings provided definite proof that the heritable defect in XP is increased susceptibility to UVL. Later it was shown that this defect is in the initial stage of the DNA repair process and may involve either lack of endonuclease (Setlow *et al.*, 1969; Cleaver, 1970) or the ability of XP cells to close endonucleolytic incisions before the occurrence of unscheduled synthesis and dimer excision (Burk *et al.*, 1971). Indeed, the defect is not restricted to fibroblasts but is also found in epidermal cells and lymphocytes (Jung, 1970). Experimentally, it is correctable by a rapidly diffusing substance from normal cells. This has been shown by *in vitro* observations in which complementation between fibroblasts from normal subjects and from patients with XP was accomplished (Giannelli, Croll & Lewin, 1972).

On the basis of the biochemical defect in XP, Regan *et al.* (1971) developed a rapid and sensitive method for its prenatal diagnosis. This is based on the observation that if cells are incubated in the presence of the thymidine analogue 5-bromo-2-deoxyuridine after UVL irradiation, the analogue is incorporated into the repair regions. If such cells are re-irradiated with UVL, breaks appear in the DNA at sites of incorporation of the analogue, leading to a downward shift in the sedimentation constant of DNA. Cells from patients with XP, because of their defect in the repair process, incorporate little of the analogue and thus the DNA sedimentation constant shows little change. Similarly, cells cultured from mid-trimester amniotic fluid in patients with XP would demonstrate the biochemical defect.

Genetic heterogeneity of XP is suggested by the clinical observation that some individuals with the classical form of the disorder have normal mentality, whereas others have some neurological abnormalities and mental deficiency. The latter type is known as the De Sanctis–Cacchione syndrome. Heterogeneity at the molecular level has been demonstrated by Bootsma *et al.* (1970) even within the classical type of XP. They studied post-UVL irradiation repair replication in fibroblast cultures by means of ^{3}H-thymidine labelling and autoradiography. Although all XP cell cultures showed decreased repair in DNA synthesis in G1 and G2 phases, this decrease was marked in cell strains originating from severe cases and slight in strains from mild cases. Cells obtained from related patients showed identical levels of repair activity, suggesting a genetically determined constant level of reduced repair replication. De Weerd-Kastelein, Keijzer & Bootsma (1972) also showed genetic heterogeneity of the two clinical forms of XP at the molecular level, by detection of intergenic complementation after application of cell fusion techniques. Binuclear cells formed by union of classic XP and De Sanctis–Cacchione skin fibroblasts, and irradiated with UVL, showed labelling patterns identical to that of UVL irradiated control cells. This meant that in these binuclear hybrid cells normal gene products were synthesized. The simplest interpretation of these observations is that two different genes are involved in the basic defect of the De Sanctis–Cacchione and classic XP, resulting in intergenic complementation.

Clinical manifestations similar to those of XP are occasionally seen in patients in their fourth decade or later. Such a condition has been referred to as pigmented xerodermoid (PX) by Jung (1971). In this condition, repair replication seems to be unaltered but there is a marked depression of premitotic DNA synthesis even after a relatively low dose of UVL. This contrasts with the observations in normal skin in which the reduction of the premitotic DNA synthesis is commensurate with the irradiation energy. It seems that in PX the first steps of the repair process involving UVL-specific enzymes such as endonuclease, exonuclease and DNA-polymerase are functionally normal, but that there may be a defect in the later steps of repair. Alternate defects may involve a DNA-polymerase system of semiconservative replication.

Patients with the classical type of XP were considered to have only skin involvement, while those with the De Sanctis–Cacchione syndrome had also neurologic abnormalities, mental retardation and hypogonadism. Recently (1972) Afifi, Der Kaloustian & Mire have demonstrated massive deposition of glycogen in the muscle of both XP and De Sanctis–Cacchione syndrome patients. The glycogen was found mainly in subsarcolemmal and interfibrillar spaces. In addition, there were subsarcolemmal aggregates of mitochondria. It is postulated that these muscle abnormalities may be due to the same genetic enzymatic defects that are responsible for the cutaneous manifestations.

The most intriguing and at the same time fascinating facet of the study of XP is the possible insight that this may give into the pathogenesis of cancer. In XP a deficiency of a single enzyme associated with the process of DNA repair correlates with the development of cancer. One can deduce that alterations in DNA may be responsible for the initiation of carcinogenesis induced by UVL in man. Cleaver (1971) hypothesizes that such a cause–effect relationship could be due to several mechanisms: increasing the rate of somatic mutations; elevating the frequency of chromosomal aberrations; multiplying alterations of DNA that would manifest themselves by an abnormal differentiation; increasing the rate of transformation of normal cells into cancerous cells by carcinogenic viruses; facilitating the expression of latent carcinogenic viruses; or accelerating cell ageing and death. Undoubtedly the problem deserves further work to understand better the enzymatic steps and molecular mechanisms that may be operative in XP. It may indeed give us a clue to the possible mechanisms of carcinogenesis.

VAZKEN M. DER KALOUSTIAN
AMAL K. KURBAN

Department of Pediatrics and
Division of Dermatology
American University Hospital
Beirut
Lebanon

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British Journal of Dermatology (1973) **88,** 517.

Correspondence

CROHN'S DISEASE WITH MOUTH AND LIP INVOLVEMENT

SIR, The paper by Stankler, Ewen & Kerr (1972) on Crohn's disease of the mouth is timely. However, I am aware of eight further cases of oral or labial Crohn's in addition to the five quoted. Thus, Schiller *et al.* (1971) described three patients with oral and one with labial disease. Another patient with oral Crohn's associated with indurated swelling of the upper lip was shown at the March 1972 Meeting of the Dermatology Section of the Royal Society of Medicine, in Oxford. (Ellis & Truelove, 1972). During a prospective study at St Bartholomew's Hospital, London (Verbov, 1973), I saw two women with the clinical appearance of oral Crohn's disease, and Professor C. D. Calnan told me recently that he has a patient with oral lesions and lip swelling.

In 1971, I saw the man with oral Crohn's described by Dudeney (1969). He then had swelling of both lips; although the disease may involve the lip itself (Schiller *et al.*, 1971) in this patient the swelling was almost certainly the result of lymphatic obstruction as a consequence of chronic buccal Crohn's.

Ordinary aphthous ulceration is common in Crohn's disease: Kyle (1972), for instance, considers that 20% of patients have such lesions at some stage during their illness. However, lip swelling and/or a hypertrophic buccal mucosa presenting a ridged, corrugated, or cobblestone appearance with or without chronic oral ulceration should be recognised as occasional signs in Crohn's disease.

JULIAN VERBOV

Department of Dermatology
University of Liverpool
Liverpool Royal Infirmary
Liverpool L3 5PU

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SEVERE RHINOPHYMA

SIR, I recently encountered a case of rhinophyma of such excessive severity that a record of it may be of interest to your readers.

His general practitioner had requested a domiciliary visit because the man, aged 83, had not ventured to leave his house for a number of years on account of a hideous deformity of his face, the central parts of which were hidden by pendulous lobes of rhinophyma.

The lowermost tip of the lesion had been continually picked by the patient causing an erosion, which bled at intervals (Fig 1).

An operation carried out by the plastic surgeon removed the deformity. An excellent cosmetic result was obtained enabling the man to resume a normal existence.

H. W. CHADFIELD

The Royal Hospital
Wolverhampton WV2 1BT

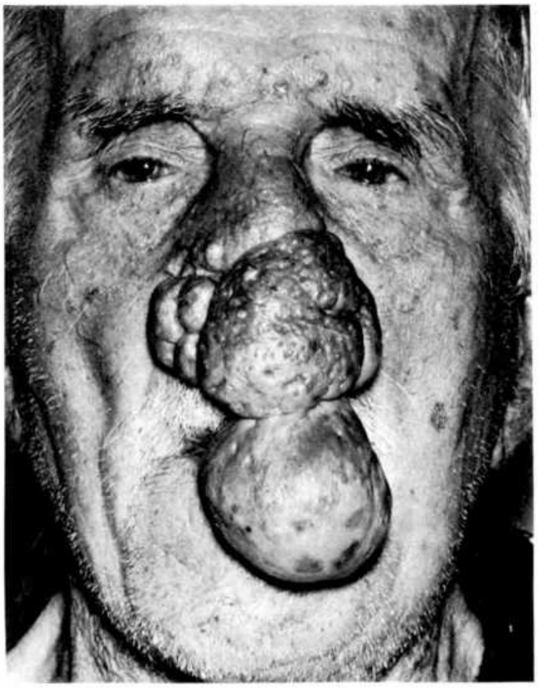

FIGURE I.

ORAL TRISORALEN IN PSORIASIS

SIR, Oddoze *et al.* (1967) found that improvement occurred in all three psoriatic patients they treated with oral meladinine followed by exposure to ultraviolet light.

Psoriasis is, of course, a splendid example of a disease characterized by remissions and exacerbations often unrelated to therapy, and these must be taken into account when the efficacy of any new therapy is assessed.

Having treated seven in-patients with widespread chronic psoriasis with oral trisoralen (4,5′,8-trimethylpsoralen) I believe that this drug is worthy of further trial in such cases. The patients, whose age range was 14–48 (mean 29·9), had previously experienced remissions lasting weeks only, even after intensive in-patient therapy in some cases. Patients received 10 mg trisoralen daily before breakfast 6 days a week over a 3-week period. One to two hours after the tablet was taken the trunk and limbs were exposed to E1 doses of ultraviolet light (preceded by a tar bath). Then, dithranol in Lassar's paste was applied twice daily to limbs and trunk (commencing with 0·1% dithranol). In five of the patients, for the first 12 days of therapy, only emulsifying ointment B.P. was applied to plaques over one upper limb and this limb was held out of the tar bath, but as lesions on this limb showed little improvement the full regime was followed for the remainder of the in-patients stay. Following the above therapy, patients were virtually free of lesions for periods varying from 5 to 11 months.

Before undertaking this study, I had in 1969 treated five patients with applications daily of meladinine paint to descaled psoriatic plaques over the left upper limb followed by exposure to suberythema doses of ultraviolet light. However, the response after 10–14 days was poor compared to that of other areas treated with dithranol in Lassar's paste and treatment with the paint was discontinued.

All of the above twelve patients before treatment had had investigations carried out, including full blood count, ESR, MSU and liver function tests. These were all normal and remained so when repeated weekly during treatment. No side-effects were experienced with the treatment.

Summarizing, the use of oral trisoralen should be considered in combination with routine topical therapy in widespread chronic psoriasis. The use of oral trisoralen and ultraviolet light without the recognised topical therapy does not hasten the resolution of psoriatic lesions, but when it was used in combination with dithranol therapy remissions were found to be more prolonged than the patients had previously experienced.

ACKNOWLEDGMENT

I thank Dr P.F.Borrie for allowing me to carry out this study at St Bartholomew's Hospital, London.

JULIAN VERBOV

Department of Dermatology
University of Liverpool
Liverpool Royal Infirmary
Liverpool L3 5PU

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UNUSUAL FEATURES OF SCABIES ASSOCIATED WITH TOPICAL FLUORINATED STEROIDS

SIR, Having read with interest the report (Macmillan, 1972) of the effect of topical fluorinated steroids on the clinical course of an infection with *Sarcoptes scabei*, I think it necessary to make some further comment and explanation than that given in the report.

Firstly the aetiology of this case is unusual in that after more than 4 months the child's mother only showed 'mildearly scabies'. This was presumably contracted by contact with the child, and can therefore be ruled out as the source, although the mother is often the origin of infant infections. Thus, the child was infected from an unknown source and must have borne the infection for some time before the initial treatment, since no erythema occurs for several weeks after infection begins (Mellanby, 1944).

Secondly, there is the effect of the steroids. Normally in scabies an immune response occurs, up to the third month, in which there is a marked reduction in parasite numbers. This is a delayed hypersensitivity-cell mediated immunity (CMI) response, probably with some skin IgE. There is polymorph infiltration and some cellular oedema in the malphigian layer (Mellanby, 1944).

In sensitivity to insect bites there is a similar response pattern (Table 1), with an 'immediate' eosinophilic reaction developing later (Benjamin & Feingold, 1970).

The prolonged application of topical steroids would have the effect of suppressing the CMI in the skin in a similar way to that described by Weston *et al.* (1972) in which, under the influence of hydrocortisone, macrophages were either rendered insensitive to macrophage migration inhibition factor or the T-lymphocytes were rendered incapable of producing it. This explains the lack of papular reaction. Any IgE response is probably also depressed. Consequently the normal pattern of very few eggs and nymphs developing to adulthood was not seen, and the population balance was upset, giving a rampant infection. The eosinophilic reaction, found at hospitalization, was due to prolonged exposure to antigenic substances and the lack of any immunosuppression of the systemic T-lymphocytes.

Immunosuppression is well known to occur with steroids and some topical preparations contain mycostatic and/or bacteriostatic compounds to reduce the risk of secondary infection. This case adequately demonstrates the very real risk, and the care required, in the use of these compounds. However,

TABLE 1. Stages of skin reactivity in response to repeated exposures to arthropod bites (after Benjamini & Feingold, 1970)

Stage	Immediate reactions	Delayed reactions
I	–	–
II	–	Infiltration by lymphocytes +
III	Infiltration by eosinophils	Infiltration by lymphocytes +
IV	Infiltration by eosinophils	–
V	–	–

here the problem also rests with the fact that no adequate attempt was made properly to diagnose the condition, even after the failure of the first treatment. This is particularly important because scabies runs in epidemic cycles (Mellanby, 1944) and is currently entering one of its peaks. In the light of this, I hope that this type of error will not be repeated.

IAN BURGESS

Department of Medical Entomology
London School of Hygiene and Tropical Medicine
Keppel Street (Gower Street)
London WC1E 7HT

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Book Reviews

World Directory of Venereal-Disease Treatment Centres at Ports. (1972) 3rd edition. Geneva: World Health Organization. Pp. 196. Price £2.40.

The World Directory of Venereal-Disease Treatment Centres at Ports was first published in 1959. A revised edition appeared in 1961. The third edition incorporates the changes that had been notified to WHO by governments up to 22 September 1971. It is divided into three main parts. The first part contains the text of the Brussels Agreement, in French, English, and Spanish, followed by lists of the countries and territories that have ratified the Agreement or subsequently adhered to it. The reservations made by some countries in respect of certain provisions of the Agreement are also reproduced. The second part of the Directory relates to the application of the Brussels Agreement and reproduces, in English and French, the technical definitions, the minimum standards, and the evaluation scheme recommended by the Thirteenth World Health Assembly. It also includes a revised model of the Personal Booklet to be issued, with instructions for use and other relevant information. The third part is the Directory proper. Information is given on the treatment facilities that are available for seamen of all nationalities at the various ports within each of the countries or territories.

The Skin. E. B. HELWIG and F. K. MOSTOFI (Ed.) (1971) London and Edinburgh: Churchill Livingstone. Pp. 618, illustrated. Price £15.50.

This monograph is the tenth in the well known series of monographs published under the auspices of the International Academy of Pathology. In the main, the various chapters are extensions of lectures given at the 57th Annual Meeting of the International Academy, for a course entitled Pathologic Physiology and Anatomy of the Skin. The intention has been to provide a permanent reference series of the subjects presented according to the 'finest scientific standards'. As might be suspected, this book has all the weaknesses of symposium publications because of the diverse aims and intentions of the various contributors who, it appears, have been given no unifying guide-line as to content, educational purposes, research and style. The result is a generally uninteresting, ill-seasoned mixed grill with some distinctly seedy ingredients. It is particularly disappointing, considering the distinction of many of the contributors. The sad truth is that in most instances the individual authors are merely rehashing work with which they have already established a name for themselves and such articles contribute nothing new. A major criticism throughout is that few references are given later than 1967; the only notable exception being in a useful article on bullous and pustular dermatoses by James Graham. On a happier note, I shall briefly mention the articles I personally enjoyed and found helpful.

Herbert Lund's chapter is a delight and an example of how not to write pompously about medical matters. He discusses among other things the so-called inverted follicular keratosis and I am glad he is not too sold on the idea of a follicular origin for this lesion.

Donald Wayte's article gives, as it were, the Armed Forces Institute view about the pathology of naevi and melanomas. This is a long article with many references (some not too up-to-date) which is generally good solid stuff. I particularly liked his discussion on juvenile melanoma.

Waine Johnson's article covers the histochemistry of the skin. He has managed to pack in a great deal of systematic information which is difficult to come by in established texts and demands a laborious search of original articles.

Wallace Clark and Rosine Bretton give details of the ultrastructure of the melanosomes in ultraviolet light irradiated skin, as compared with those found in the abnormal cells of lentigo maligna and superficial melanocarcinoma. Their study can be commended.

Edwin Fisher and Harry Wechsler cover a rather wide field in their chapter on the collagen diseases and elastoses. It is written from a different point of view to the straightforward dermatological one and much interesting information emerges.

Hermann Pinkus (Embryology and anatomy of skin) and Richard Winkelmann (New methods for the study of nerve endings) are their usual elegant selves.

E. WILSON JONES

Advances in Microcirculation. Vol 4. H. HARDERS (Ed.) (1972) Basel: Karger. Pp. viii+233, 87 figures, 45 tables. Price: £9.60.

This is the fourth of a series of very expensive, well printed books which were originally intended to supplement *Bibliotheca Anatomica:* records of the Biennial International Conferences organized by the European Society for Microcirculation. The latter are now recorded in symposium editions, together with separate less detailed proceedings.

The Advances in Microcirculation series provides an opportunity for full documentation of techniques and results. This volume describes only six topics. The first paper suggests that haemodilution with dextran 60 is a satisfactory therapy of all microcirculatory disorders due to a decreased fluidity of blood. However, there are already more up to date reviews by the same workers to be found in *Haemodilution,* *Theoretical Basis and Clinical Application*, K. Messmer and H. Schmid-Schonbein (Ed.), (1972) Basel: Karger.

In the second paper, hamster experiments are described which show that cream depressed corpuscular flow velocity to a greater extent than vegetable oils, but the reason for this remains unknown.

A paper by Asano & Branemark describes their titanium chamber. This is inserted into a tube pedicle in the skin of the upper arm of human volunteers and is a means of observing subcutaneous blood vessels *in vivo* in man. The microcirculation can be accurately recorded. The fourth paper describes experiments in rabbits, in which damage to the skin from freezing is minimized when rapid rewarming is up to temperatures not exceeding 42°C. The fifth paper discusses the use of tritium tagged foreign colloids. The final paper, describing leukocyte sequestration in the lung in shock, may be the most interesting to dermatologists because of its relevance to leukocytoclastic angiitis. The behaviour of leukocytes in areas of stasis and protection by prednisolone is illustrated by this electron microscopic study.

The book, though glossy, contains a great number of very poorly construed sentences. Could not Karger insist on the correction of their manuscripts by someone who has a complete command of the English language?

T. J. RYAN

Haut als endokrines Erfolgsorgan; Gestagene; geriatrische Endokrinologie des Mannes. J. KRACHT (Ed.) (1971) Berlin, New York: Springer. Pp. 196+vii, 84 illustrations, 7 plates. Price: £4.75.

Part of the 17th symposium of the German Society for Endocrinology was devoted to hormone action on skin. Mainly androgens were discussed. In these proceedings, Dr Jean Wilson, from Dallas, U.S.A., describes testosterone metabolism in skin with special reference to formation of dihydrotestosterone. The latter is probably involved critically in the androgen-stimulated growth and differentiation of human skin. The 5 α-reductase system, responsible for converting testosterone to the dihydro form is also characterized. Dr F. J. Ebling, of Sheffield, England, gives a more general account of steroid action on skin, mainly on the sebaceous glands of the experimental animal. Only these two contributions to the symposium are of considerable value to the dermatologist and would interest those working in the field of acne.

As regards 'geriatric endocrinology of the male', the suspicion that androgen activity might lessen with age is confirmed in the papers of these proceedings.

MICHAEL FEIWEL

Theses and Disertations

Dermatitis herpetiformis: specific (immunopathological?) entity. *A histopathological and immunofluorescence study of fifty-nine patients with dermatitis herpetiformis and parapemphigus (pemphigoid)*. J. B. VAN DER MEER (1972) Doctoral thesis, Utrecht. Pp. 121, 42 illustrations. N. V. Boekhandel V.h. C. Kooyker, Universiteitsboekhandel, Leiden.

Diseases of unknown aetiology are diagnosed when defined criteria are met. This thesis examines possible diagnostic criteria for dermatitis herpetiformis by reviewing literature and from the findings in a group of patients for whom investigations are reported. Four criteria are described. The review of literature reveals much confusion starting from the description of many diseases as dermatitis herpetiformis by Duhring and as dermatites polymorphes douloureuses by Brocq. Since then, despite an objecting minority, dermatitis herpetiformis and pemphigoid have been separated as distinct entities, but diagnostic criteria have varied. Van der Meer has analysed the clinical, histopathological and immunohistochemical features of his group of fifty-nine adult patients. Unfortunately all were not treated with dapsone, sulphapyridine or related drugs, but response to these drugs is a major criterion for the diagnosis of dermatitis herpetiformis. All patients finally diagnosed on the basis of the other criteria as having dermatitis herpetiformis, who were treated with these drugs, showed a good response.

The second clinical criterion for diagnosis from pemphigoid is the distribution of the rash which, in dermatitis herpetiformis, selectively involves the elbows, shoulders and scapular area, buttocks and sacral area and knees.

Histological examination of skin biopsies, with in addition specimens from ten patients with erythema multiforme, confirmed that neutrophil papillary micro-abscesses are a reliable third diagnostic criterion for dermatitis herpetiformis. In the variety of pemphigoid with much erythema, occasional papillary micro-abscesses were found but these contained almost exclusively eosinophils. In a few biopsies from patients with dermatitis herpetiformis the papillary micro-abscesses contained eosinophils but these were never the predominant cell type. Histologically, erythema multiforme was distinct with a lymphocyte infiltrate about dermal blood vessels showing endothelial swelling and at times lymphocyte invasion of epidermis and appendages. Polymorphs and eosinophils were rare.

The fourth diagnostic criterion was provided by immunohistochemical examination of uninvolved skin. In dermatitis herpetiformis this showed deposits of immunoglobulin in a granular pattern in the basement membrane zone with concentration of deposit at the apex of dermal papillae. The deposits were predominantly of IgA. The presence of these deposits was not influenced by treatment with dapsone or related drugs. The linear deposits predominantly of IgG in pemphigoid showed a clearly different pattern. An additional three patients with herpes gestationis showed the immunofluorescent pattern found in pemphigoid.

For each of the four diagnostic criteria less than 10% of patients gave results inconsistent with the result obtained by combining findings from the other three criteria. This rate of error could be abolished for the first criterion—that of drug responsiveness—by adopting this in the definition of dermatitis herpetiformis. But each criterion is as consistent as the others and dapsone with related drugs are far from specific for dermatitis herpetiformis. The distinction between dermatitis herpetiformis, pemphigus and erythema multiforme is readily made and can be confirmed histologically. Pemphigoid, in which bullae arise on skin which is otherwise uninvolved clinically, presents few difficulties. In pemphigoid with small and large bullae arising on a background of erythema, clinical and histological criteria may be inconclusive and immunohistochemical examination of uninvolved skin may be required to obtain a diagnosis.

Van der Meer has examined the integrity of dermatitis herpetiformis as a specific entity separated from the group of erythemato-bullous disorders of Duhring and Brocq. While some of his findings have previously been reported in studies on small groups of patients this careful study of a large group of patients provides further clear evidence that a group of criteria serves to segregate dermatitis herpetiformis as a distinct disease pattern.

A. L. MACMILLAN

British Journal of Dermatology (1973) 88, 524.

News and Notices

British Journal of Dermatology

All enquiries regarding subscriptions to the *British Journal of Dermatology* should be sent to the Publishers, Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL.

First International Symposium on Paediatric Dermatology

This Symposium, which is designed for paediatricians, general practitioners and dermatologists, will be held at the Hospital del Niño I.M.A.N. in Mexico City, on 17–20 October 1973. Further information may be obtained from Dr Ramón Ruiz-Maldonado, Department of Dermatology, Hospital Del Niño I.M.A.N., Insurgentes Sur 3700–C, Mexico 22, D.F., Mexico.

International Health Foundation Award—1973

An award of 5000 Swiss Francs will be presented to the author of the best entry submitted to the International Health Foundation in Geneva on: 'The control of venereal disease'.

An additional prize of 2000 Swiss Francs is available for the best entry by an author under 35 years of age.

The closing date for entries is 1 September 1973, and further details regarding the panel of judges, technical requirements and so on may be obtained from the International Health Foundation, 1 place du Port, 1204 Geneva, Switzerland.

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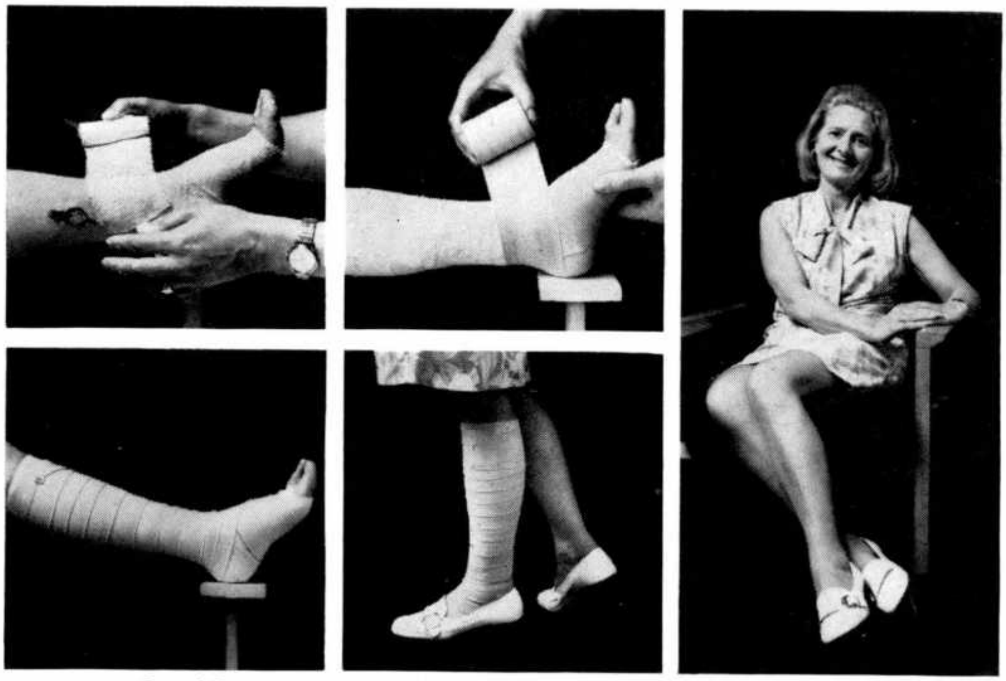

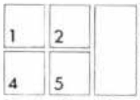

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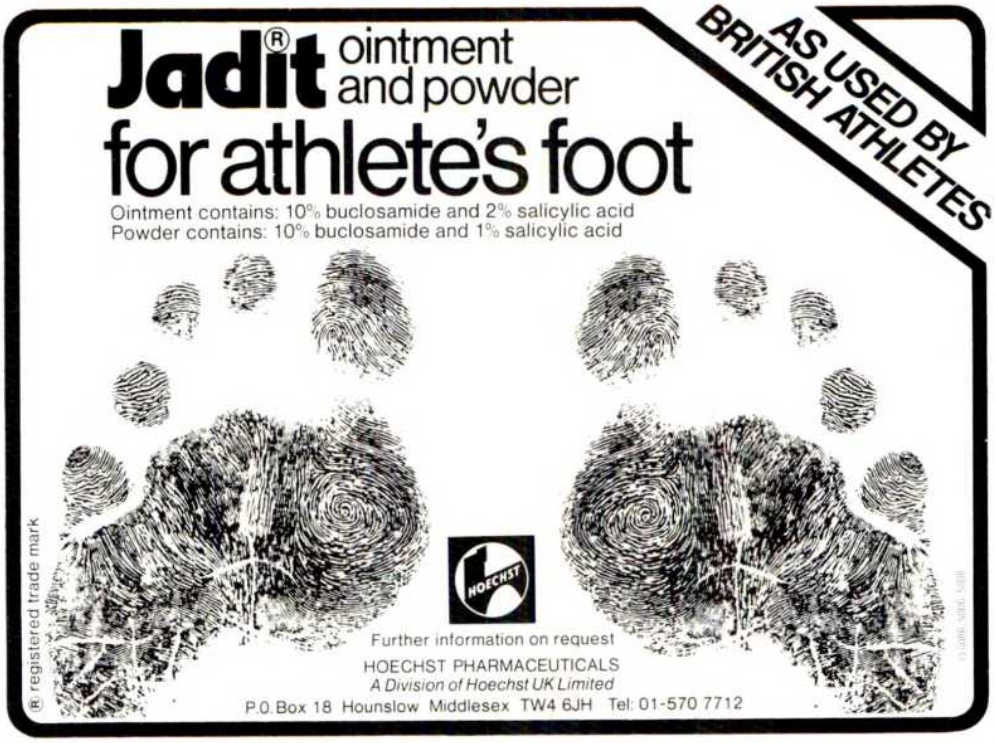
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F.J.G. Ebling D.SC. PH.D.

The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this edition is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

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John Martin Beare M.D.(Belfast) F.R.C.P.(Lond.)
Robert John Cairns M.B. B.S.(Lond.) F.R.C.P.(Lond.)
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nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	percent	%
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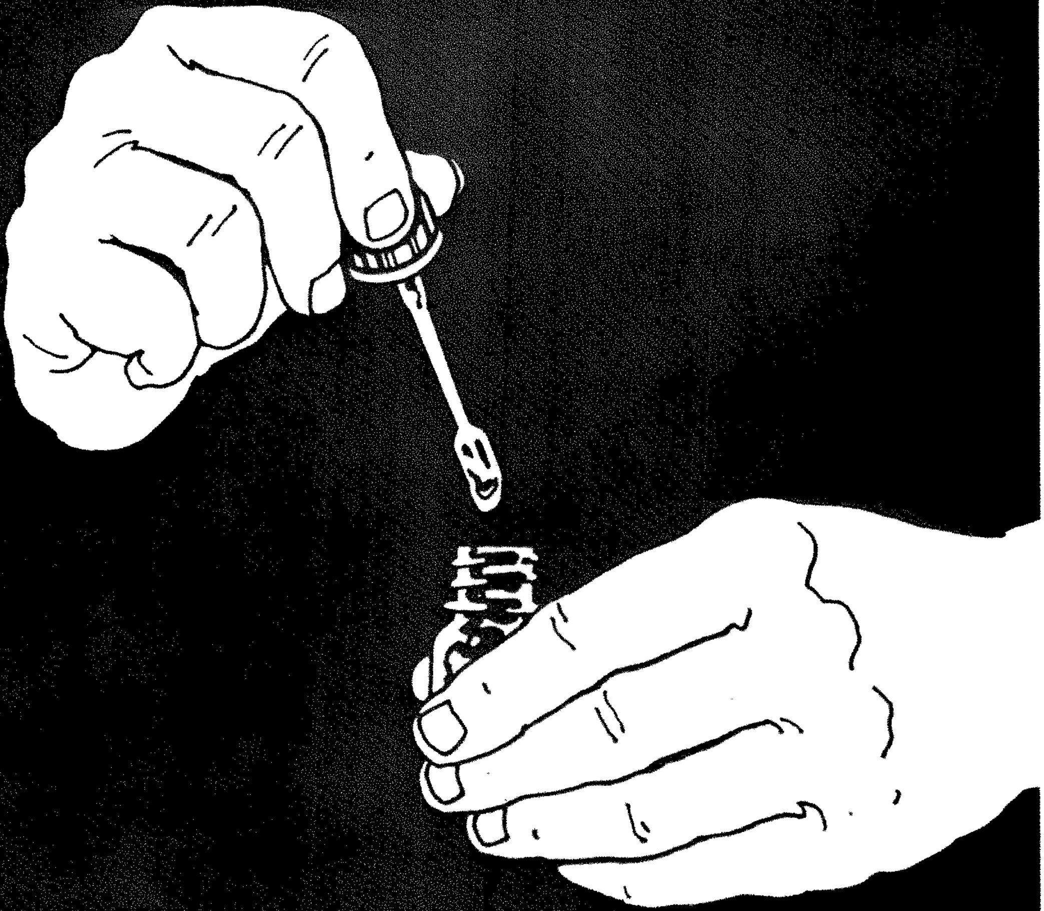

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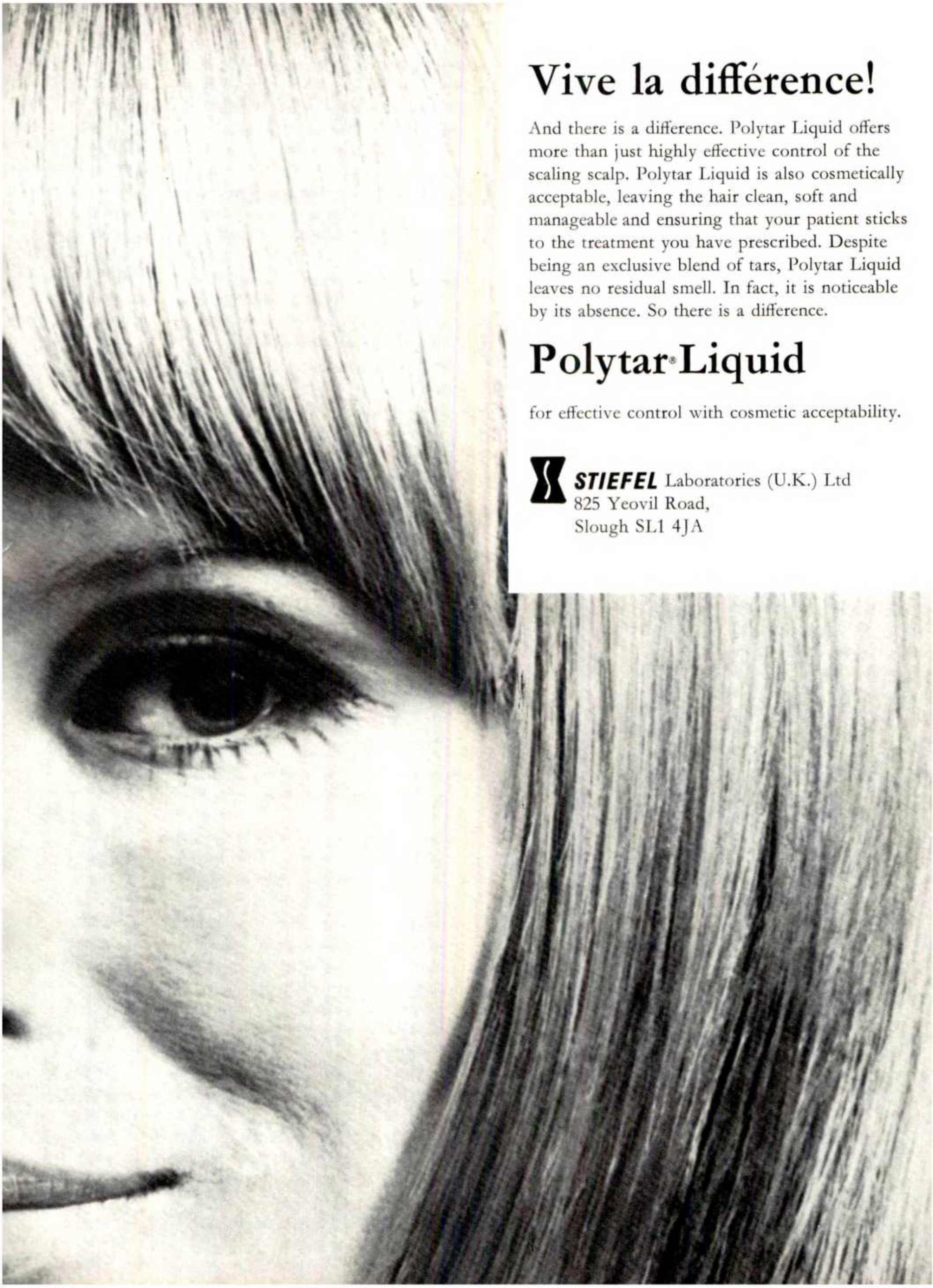

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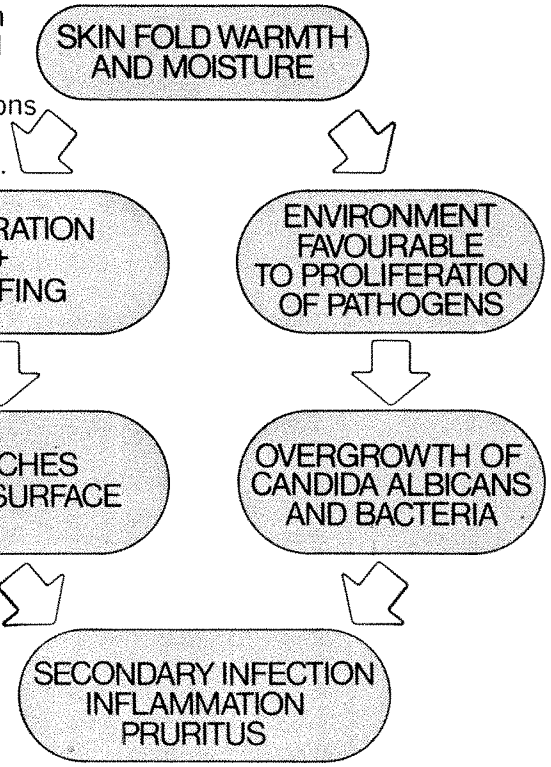
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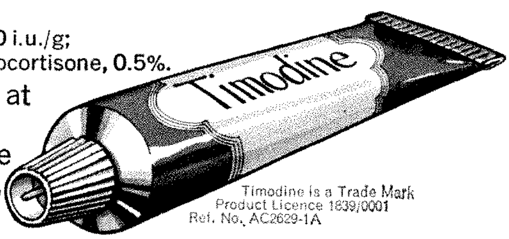

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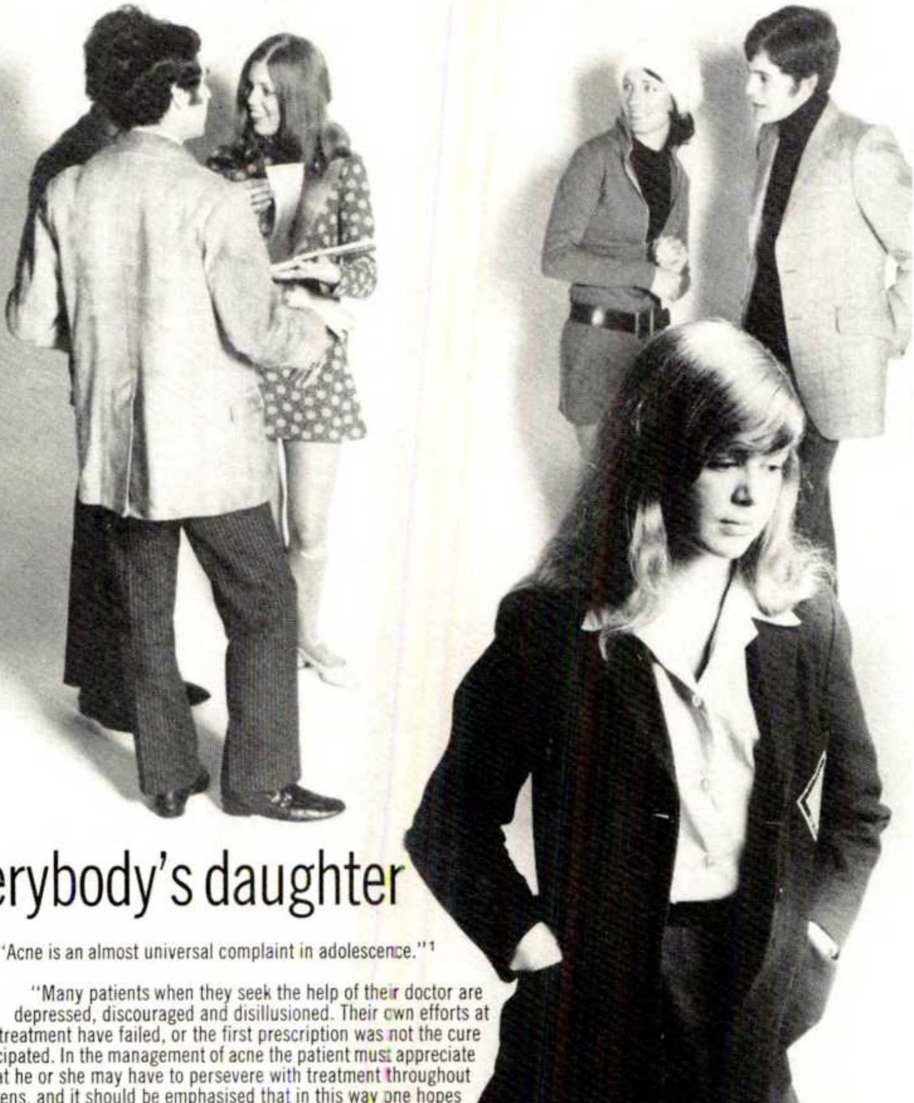

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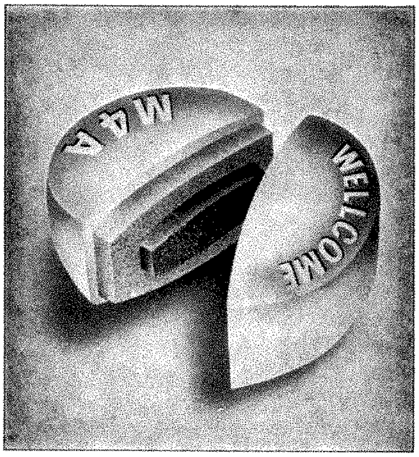

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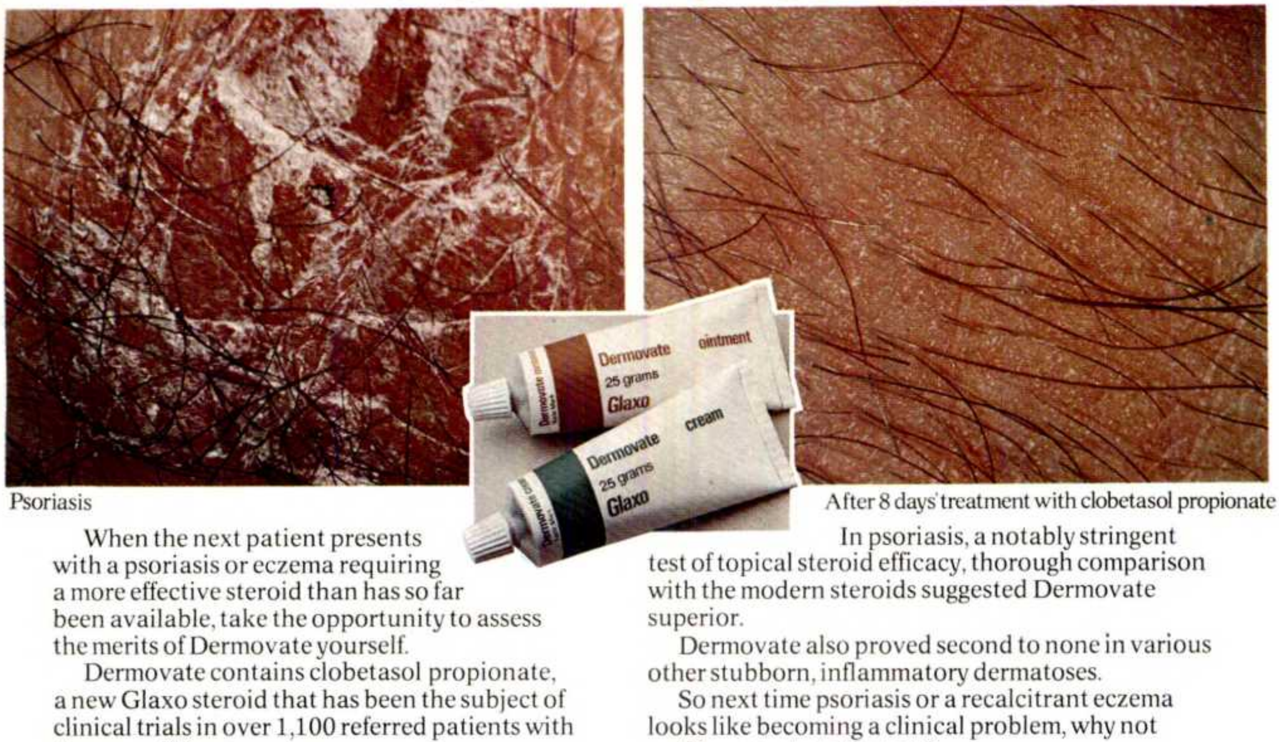

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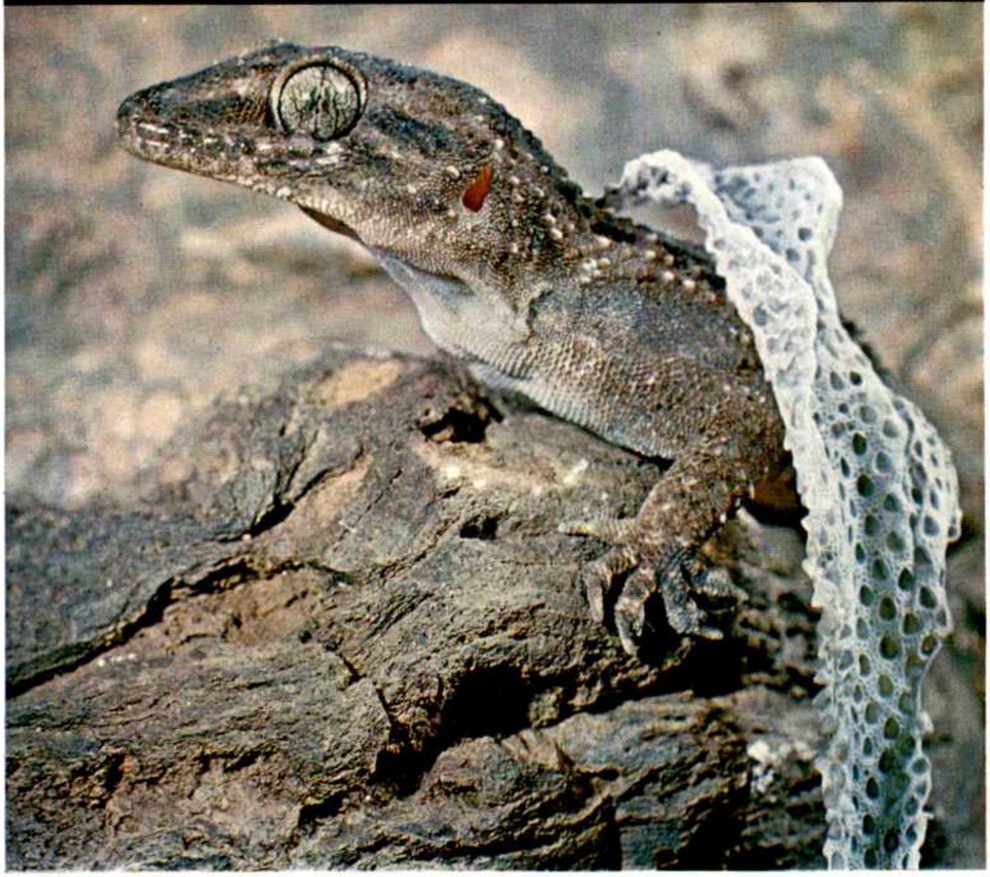

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Clinical and Laboratory Investigations

Urticaria induced by preservatives and dye additives in food and drugs

GERD MICHAËLSSON AND LENNART JUHLIN

Department of Dermatology, Uppsala University Hospital, Uppsala 750 14, Sweden

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SUMMARY

Fifty-two patients with recurrent urticaria or angio-oedema and thirty-three controls have been provoked with five different food dyes and the preservatives sodium benzoate and 4-hydroxy-benzoic acid, as well as aspirin, sulphanilic acid and a placebo. The reaction was judged as positive in thirty-nine patients who developed urticaria within 14 h. Of these, thirty-five reacted to aspirin, twenty-seven to benzoic acid compounds and twenty-seven to azo dyes. The four patients who did not have urticaria after aspirin, reacted with urticaria to benzoic acid compounds, and three of them to azo dyes. No definite pattern for the reaction to the different azo dyes was seen. None had an urticarial reaction from sulphanilic acid, Patent Blue (a non-azo dye) or placebo. The doses of additives used in the provocation tests are easily exceeded in daily life by the consumption of foods and drugs. Recurrences of urticaria could be prevented through the avoidance of food and drugs containing azo dyes and preservatives.

Aspirin-sensitive patients sometimes react adversely to other analgesics, like the indol and pyrazolon derivatives, and to azo dyes such as tartrazine. Reactions to tartrazine were first described by Lockey (1959). Since then about twenty-five such cases have been described (Juhlin, Michaëlsson & Zetterström, 1972). Samter & Beers (1968) found that about 8% of their aspirin-sensitive patients also reacted to tartrazine. In a study of reactions in aspirin-sensitive patients, we found that seven out of ten patients with recurrent urticaria and/or asthma reacted to tartrazine. Furthermore, seven of them also reacted to benzoic acid and some of its derivatives (Juhlin *et al.*, 1972). The high frequency of reactions to these common food and drug additives has prompted us to extend with several dyes the provocation tests in patients with recurrent urticaria and angio-oedema. This paper reports the results obtained in a group of fifty-two patients with recurrent urticaria. Examples of food with and without azo dyes, benzoates and salicylates are also included.

Patients

Fifty-two patients with symptoms of recurrent urticaria and angio-oedema were tested. No patients with urticaria factitia, cold or heat urticaria were included in the group. All except five cases were investigated on the wards. The medical histories indicated the presence of an aspirin hypersensitivity in only seventeen patients. Five of the patients also had asthma. Several patients had had urticaria periodically or continually for years. In some of the cases, the symptoms had been so intense that they required long periods of sick leave and hospital care. All of the patients had been treated with different antihistamines, mostly without any improvement. Sometimes an unexplainable worsening had been noted after certain antihistamine tablets which were coloured.

The sampling of patients is, to some extent, selective. Twelve of the patients had visited the dermatology clinic earlier and had been found to be sensitive to aspirin but had, despite avoidance of this medication, continued to have urticaria. The other patients had visited the clinic for the first time for recurrent urticaria of unknown aetiology during the period December 1971–November 1972.

Controls

Thirty-three healthy persons served as controls. Two of them have a moderate allergic rhinitis and one sometimes has an itching of unknown cause.

TABLE 1. Foods which often contain azo dyes and benzoic acids

(a) *Azo dyes*

Penny candies, caramels and chews, life savers and fruit drops, filled chocolates, but not pure chocolate.

Soft drinks, fruit drinks and ades.

Jellies, jams, marmalades, stewed fruit sauces, fruit gelatines, fruit yoghurts, ice cream, pie fillings, vanilla, butterscotch and chocolate puddings, caramel custard, whips, dessert sauces, such as vanilla, and cream in powdered form. Bakery goods except plain rolls, crackers, cheese puffs, etc., chips, cake and cookie mixes, waffle/pancake mixes, macaroni and spaghetti (certain brands).

Mayonnaise, salad dressings, catsup (certain brands), mustard, ready made salads with dressings, remoulade, bearnaise and hollandaise sauces, as well as sauces such as curry, fish, onion, tomato and white cream.

Mashed rutabagas, purées, packaged soups and some canned soups.

Canned anchovies, herring, sardines, fish balls, caviar, cleaned shellfish.

Colored toothpastes.

(b) *Preservatives containing benzoic acid compounds*

Soft drinks, ciders, fruit drinks and ades.

Jellies, jams, marmalades, fruit gelatines, stewed fruit sauces.

Cheese, especially cream cheeses, low-calorie margarines, salad dressings, remoulade, hollandaise, bearnaise and mustard sauces, and readymade salads with dressings.

Refrigerated preserves of herring, sardines, anchovies, shellfish and fish.

Surface-treated fish (can be rinsed away).

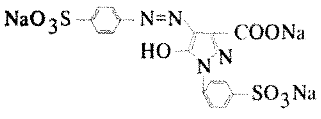
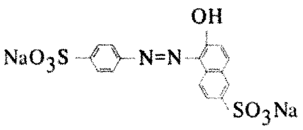
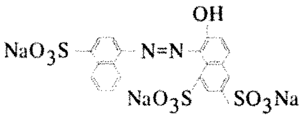
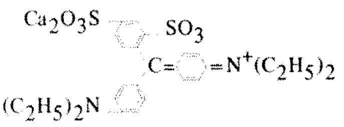
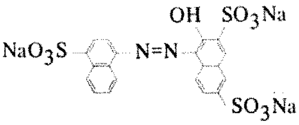
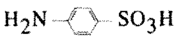
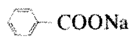
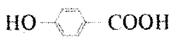
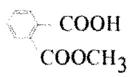
Diet and drugs during the testing period

For 3 days before admission to hospital, the patients were requested not to use antihistamines or any other drugs, if possible. The patients with frequent recurrences of urticaria had been on a diet free from dyes and preservatives before being admitted. All patients were on such a diet while in the hospital. Examples of food without additives which we have found that patients with aspirin hypersensitivity usually can tolerate are: bread, cereals, rice, sugar, potatoes, butter, salad oils, eggs, milk, cream, meat, including chicken and turkey, fish, lettuce, parsley and mushrooms. They are recommended to drink only plain water. The types of food products containing additives which should be avoided are shown in Table 1. Small amounts of naturally occurring benzoates and salicylates have been found, especially in blue berries, lingon berries, bananas, green peas and licorice (Juhlin & Michaëlsson, 1973). Many aspirin-sensitive patients do not tolerate rhubarb, grapes, apples, European red wines and beers, although we have not been able to demonstrate the presence of benzoates or salicylates here. Other fruits and vegetables are allowed one at a time after a period on the basic diet. Inquiries concerning the amount of dyes and benzoates in drugs have been sent to all drug companies represented in Sweden. The information received reveals that one tablet can often contain 0.01–3 mg of an azo dye. Drugs free from these compounds have been listed, and are the only drugs allowed for these patients (Juhlin & Michaëlsson, 1973). Aspirin, as well as other anti-inflammatory analgesic drugs, are not allowed. If an analgesic drug has to be given, paracetamol might be tried, although cross reactions have been described for this drug as well (Smith, 1971).

Substances used for provocation

The doses, structural formulae, and colour indices (CI) of the substances used in the provocation tests are shown in Table 2. The substances were given in colourless gelatine capsules, with lactose added to complete the filling.

TABLE 2. Substances and doses used for provocation.

Substance	Dosage (mg)	Structural formula
Placebo (Lactose)	100	
Tartrazine CI 19140 Colour: yellow	0.1* 1, 2, 5, 10	
Sunset Yellow CI 15985 Colour: yellow-red	0.1,* 1, 2, 5, 10	
New Coccine CI 16255 Colour: red	0.1,* 1, 2, 5, 10	
Patent Blue V CI 42051 Colour: blue	5	
Amaranth CI 16185 Colour: red	0.1,* 1, 2	
Sulphanilic acid	50	
Sodium benzoate	50, 250, 500	
4-hydroxybenzoic acid	50, 100	
Aspirin	0.1,* 1, 5, 10 50, 500, 1000	

* Initial doses which have been used for those with a history of asthma.

TEST METHOD

Provocation tests were performed when the patients had slight or no symptoms. After a light breakfast, the substance to be tested was given in the lowest dose at 8 a.m. If no objective reaction could be noted, additional and increased doses were given at 1h intervals. When a positive reaction occurred, the provocation was stopped and no further doses were given. Only one substance was given per day. The doses which were usually used are shown in Table 2. The patients with a history of asthma began with the lowest dose. As a rule, we used the placebo first and ended with aspirin. The provocation tests which were questionable and difficult to interpret were usually repeated on another day. The next test was not made before the previous reaction had disappeared. If asthmatic symptoms developed, adrenaline was given so that the reaction was not prolonged. No therapy was given for urticaria.

Both objective and subjective symptoms were carefully noted after each test. The symptoms which developed are seen in Table 3.

TABLE 3. Symptoms of a hypersensitivity reaction

Objective signs	Subjective symptoms
Urticaria	Itchy skin
Angio-oedema of the lips, eyelids or face	Eye irritation
Reddening of the eyes	Nasal congestion
Sweating	Breathing difficulties
Increased tear secretion	Irritability
Nasal congestion	Upset stomach
Sneezing	Hot flushes
Rhinitis	Sensations of:
Hoarseness	Swollenness
Wheezing	Tiredness
	Drowsiness
	Thirst
	Stinging in the lips and throat
	Pressure across forehead
	Heaviness in the head

Some of the patients who had severe reactions after a provocation did not want to continue with the tests as planned; therefore, it has not been possible to carry out tests with all compounds in every patient.

Evaluation of symptoms

The provocation results were judged as positive when the patient developed an unquestionable urticaria or angio-oedema after having had an inactive period before provocation. The reaction, especially angio-oedema, sometimes occurred within the first few hours after provocation, whereas the urticaria often did not develop until 6–14 h afterwards.

Other objective symptoms which often appear during the first hours, but which can easily be overlooked, include erythema of the neck and face, nasal congestion, sneezing, hoarseness, and wheezing—these are listed in Table 3. In some cases they occurred without urticaria or angio-oedema, as shown in Table 4 under the heading; 'Other objective symptoms'. Such provocation results have been designated by us as 'probably positive'.

Subjective symptoms which have been difficult to verify are, for example, itching and a sensation of being swollen or warm (Table 3). Most patients who developed urticaria also mentioned these symptoms. The number of patients who had no objective changes, but who mentioned one or several subjective symptoms, is shown in Table 4. Their provocation results have been classified by us as 'probably negative'. Comparison of the results from a placebo is essential, however, and may be of help in the evaluation.

RESULTS

Thirty-nine of the fifty-two patients tested had positive reactions to one or several substances. The results of the provocations of these thirty-nine patients are summarized in Table 4. All except four of the patients reacted to aspirin with urticaria. The total amount of aspirin which produced urticaria was 1–10 mg in three patients, 20–200 mg in eleven, 400–700 mg in eleven, and 800–1500 mg in ten of them. The four patients without symptoms after aspirin had reactions to one or both benzoic acid compounds and to one or several dyes (Table 5). The histories and repeated provocation with a single dose of 1000 mg of aspirin gave no support for aspirin hypersensitivity in these patients.

Twenty-two of thirty-seven patients reacted with urticaria to sodium benzoate and twenty-one of them to 4-hydroxy-benzoic acid (Table 4). Sixteen developed urticaria to both substances while eleven had urticaria from one or the other. Seven patients had no objective symptoms from any of the benzoates. In all except three, the highest specified dose was given.

TABLE 4. Results of provocation tests. The figures show the number of patients. Of the fifty-two patients, thirty-nine reacted with urticaria to one or several provocations. None of the thirteen patients who did not have any objective signs are included

Substance used	Urticaria	Other objective signs (Table 3)	Only subjective symptoms	No reaction	Total no. tested
Placebo	0	0	4	35	39
Tartrazine	19	2	1	17	39
Sunset Yellow	10	0	6	11	27
New Coccine	9	1	6	9	25
Amaranth	1	1	3	2	7
Patent Blue V	0	0	2	17	19
Sulphanilic acid	0	0	2	6	8
Sodium benzoate	22	2	3	10	37
4-OH-benzoic acid	21	3	4	9	37
Aspirin	35	0	0	4	39

Tartrazine caused urticaria in nineteen patients (Table 4). About two-thirds of those reacting also had urticaria after other azo dyes and benzoic acid compounds (Table 5). Fifteen of the eighteen patients who were negative to tartrazine were tested with one or several other dyes. Seven of them reacted with urticaria to other azo-dye substances. In Table 5 the individual reactions have been listed for twenty-three patients tested with benzoic acid compounds and four dyes. No definite pattern for the reactions to the different azo dyes was observed. The degree of the reactions varied; in some cases the reactions were severe after 1–2 mg of dye, and even more pronounced than after 500 mg of aspirin. In all, twenty-seven patients reacted to some azo dye. In twelve of the patients, urticaria developed after 1–3 mg of tartrazine, while in the others 5–18 mg was needed. The doses of the other azo dyes causing urticaria were of the same magnitude. None of the patients reacted to Patent Blue, which is a non-azo dye, or to sulphanilic acid, which is a metabolite of some azo dyes.

Placebo tablets gave no objective symptoms in any of the patients. Subjective symptoms were noted in four patients after the placebo. In patients with only subjective symptoms after the test substances, the number and types of symptoms did not in any case differ from those reported after the placebo.

Aspirin hypersensitivity was suspected from the medical history of three of thirteen patients with negative provocation tests. The three patients improved on a diet free from salicylates and dyes. In the other ten patients, we found no reason for the urticaria.

TABLE 5. Comparison of the individual reactions to provocations with placebo, aspirin and five common food and drug additives

Patient	Placebo	Aspirin	Sodium-benzoate	4-OH benzoic acid	Tartrazine	Sunset Yellow	New Coccine	Patent Blue V
1	o	+	+	+	+	+	+	o
2	o	+	+	+	+	+	+	o
3	o	+	+	+	+	o	o	o
4	o	+	+	+	+	o	o	o
5	o	+	+	+	o	+	+	o
6	o	+	+	+	o	+	+	o
7	o	+	+	+	o	o	o	o
8	o	+	+	o	o	+	o	o
9	o	+	+	o	o	o	o	o
10	o	+	+	o	o	o	o	o
11	o	+	(+)	o	+	o	+	o
12	o	+	o	+	+	+	o	o
13	o	+	o	+	+	+	o	o
14	o	+	o	+	o	o	o	o
15	o	+	o	(+)	o	+	+	–
16	o	+	o	o	+	o	o	o
17	o	+	o	o	(+)	o	o	–
18	o	+	o	o	o	o	+	–
19	o	+	o	o	o	o	o	o
20	o	o	+	+	o	o	+	o
21	o	o	+	+	o	o	+	o
22	o	o	+	(+)	(+)	o	o	o
23	o	o	+	o	+	+	(+)	–
Total +	o	19	14	12	9	9	9	o
Total (+)	o	o	1	2	2	o	1	o
Total o	23	4	8	9	12	14	13	19

o, no reaction; (+), other objective signs; –, provocation not done; +, positive reaction with urticaria or angio-oedema.

None of the thirty-three subjects used as controls developed urticaria. Two subjects, a mother and her son, with a history of moderate allergic rhinitis showed signs of rhinitis after tartrazine and Sunset Yellow, but not after repeated doses of aspirin. Subjective symptoms, but no objective ones, were noted in three of the controls. They occurred after placebo, sodium benzoate, tartrazine and New Coccine.

DISCUSSION

Provocations were only done with some of the most common food additives. Several other accepted dyes have not yet been investigated; nevertheless, the number of patients who reacted with urticaria after provocation was quite large. An investigation of asthma patients in the Pulmonary Clinic of the University Hospital gave similar results (Rosenhall & Zetterström, 1973).

Neither the mechanism behind hypersensitivity reactions to aspirin nor the cross reactions in aspirin-sensitive patients to analgesic drugs, such as pyrazolone and its derivatives, are well understood. The results of provocation tests in our patients with urticaria demonstrate that these patients not only react to aspirin but also to benzoates and to commonly used azo dyes. One possible mechanism for cross reaction between pyrazolone and tartrazine might be that the azo-reductive cleavage of tartrazine in the intestines produces, in addition to sulphanilic acid, 1-(4-sulphophenyl)-3 carboxy-4-amino-pyrazolone (Ryan, Welling & Wright, 1969). This can later be hydrolysed to 4-sulphophenylhydrazine. We have not yet tested any of these compounds. For some of the azo dyes, a formation of aniline compounds can be shown (Walker, 1970). This could be an explanation for the cross reaction with the tested preservatives.

A variability in response to various analgesics in aspirin-sensitive patients has been reported by Smith (1971). We also found that the pattern for reactivity to the substances tested varies markedly from one patient to another. We have no explanation for this. It seems that no definite conclusions can be drawn from a single negative provocation. One might speculate that some tests have been performed too soon after a positive test, when the patient is still in a refractory state, or there might have been symptoms and signs which have been overlooked, or inappropriate test doses might have been used. Another explanation to the sometimes inconsistent pattern is that, despite the diet, the patient might have shown a possible reaction to some unknown antigen or might have been in a state of increased reactivity after a preceding provocation. Against such an assumption is the negative reaction to the placebo, Patent Blue and sulphanilic acid.

The doses used for provocation are in the amounts which can easily be consumed during a day, with food or medicine. The choice of provocation dose is based upon our earlier experiences. Thus, 4-hydroxybenzoic acid gave a stronger reaction than sodium benzoate in the same dosage; therefore, the former has been given in lower doses in our tests and the results of the provocations are not completely comparable. In several countries both compounds are approved for use in foods in the same amounts.

Since some of the patients did not have any symptoms until 6 h after provocation, theoretically it might be more correct to have a 6 h interval between the doses; however, for practical reasons, shorter intervals were preferred.

The majority of the patients with a hypersensitivity to dye additives also reacted to aspirin. The reactions after aspirin were often pronounced; therefore, this test should be carried out at the end of the investigation, or with a separate provocation a couple of weeks before the others. Negative aspirin provocation does not, however, exclude a sensitivity to additives. Thus, four of our patients repeatedly reacted only to additives and not to aspirin. Some patients also had a stronger reaction to dyes than to aspirin. The fact that two controls also developed nasal congestion after tartrazine and Sunset Yellow but no symptoms after repeated provocations with aspirin, may indicate that hypersensitivity to additives without a parallel sensitivity to aspirin is more common than believed. The patients who were found to be hypersensitive to aspirin were often not aware of this. Only seventeen of our fifty-two patients had correlated their symptoms with aspirin consumption. One explanation may be that some of them had had urticaria a long time before they had taken any salicylic product and noticed a worsening even without aspirin consumption.

The high frequency of hypersensitivity reactions to food additives can possibly explain why the patient's urticaria often recurs. In many patients with recurring urticaria of a previously unknown cause, we have now, after positive provocation tests, been able to correlate the exacerbations of the urticaria with the intake of food and drugs containing certain additives. A dietary regimen is therefore recommended. Sixteen patients with positive provocations have been kept on a diet without dye additives or preservatives for 6 months, and thirteen of them have been completely free from urticaria, one has obviously improved, and two continued to have urticaria.

Information concerning the occurrence of these common additives in both food and medicine is difficult to obtain. Specifications of dyes and preservatives in medicines are lacking. The care of patients sensitive to additives is impossible, however, without this information. Prescription of unsuitable medicines has undoubtedly contributed to the continuation of the disease in several patients. Therefore, on the basis of information obtained after inquiries, lists of Swedish food and drugs which are free from additives have been published (Juhlin & Michaëlsson, 1973). Types of foods which are common in most countries and often contain azo dyes and preservatives are exemplified in Table 1. Lack of information has, in all probability, contributed to the delay in realizing that these substances had a damaging effect for a relatively large group of patients.

ACKNOWLEDGMENT

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Gram-negative folliculitis—a complication of antibiotic therapy in acne vulgaris

JAMES J.LEYDEN,* RICHARD R.MARPLES, OTTO H.MILLS, JR
AND ALBERT M.KLIGMAN

Department of Dermatology, University of Pennsylvania School of Medicine, 3400 Spruce Street, Philadelphia,
Pennsylvania 19104

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SUMMARY

Fifty cases of Gram-negative folliculitis have been identified amongst 1200 new cases of acne vulgaris in a period of 4 years. Two clinical varieties are recognized. Type I, the more common, presents as superficial pustules, usually grouped around the nose and is associated with a lactose fermenting Gram-negative rod. Type II lesions are characterized by deep nodular and cystic lesions; *Proteus* appears to be the causative agent. The treatment of this condition is discussed.

There has recently been a world-wide increase in systemic infections caused by Gram-negative organisms (Findland, Jones & Barnes, 1959; Barber, 1961; Johnstone, 1970; Adler, Martin & Findland, 1971). The widespread use of antibiotics effective predominantly against Gram-positive organisms has been incriminated as the probable cause for this shift. We previously reported a cutaneous example of antibiotic induced superinfection; namely, Gram-negative folliculitis complicating the treatment of acne vulgaris (Fulton *et al.*, 1968). This syndrome consisted of the following: (1) an older aged acne patient who had been on antibiotics, usually tetracyclines, for prolonged periods, (2) pustular lesions grouped about the nose and fanning out on to the cheeks, (3) the recovery of Gram-negative lactose fermenting rods from the anterior nares and from one or more of the pustules, (4) histological evidence of an intrafollicular abscess without comedone formation and (5) clinical improvement following antibiotic therapy directed against the Gram-negative organisms.

Our experience since has broadened to fifty patients. It is our intent in this report to delineate this syndrome from the viewpoint of diagnosis, pathogenesis and treatment.

METHODS AND MATERIALS

The patients were gathered from the Acne Clinic of the Hospital of the University of Pennsylvania. It is the policy of the clinic to take cultures from any new patient referred as a 'resistant' case and any patient with a predominance of pustular or cystic lesions. The technique of culture was as follows:

The lesion was wiped with an alcohol sponge and then penetrated with a sterile hemolet to collect a drop of pus. This was placed in 0·5 ml of buffered Triton-X-100 wash solution. The anterior nares were sampled by rotating Triton moistened applicators twenty times within the nose. These samples were then processed and cultured on multiple media as previously described (Williamson & Kligman, 1965; Marples *et al.*, 1969).

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* Reprint requests to: Dr Leyden, Department of Dermatology, Duhring Laboratories, Hospital of the University of Pennsylvania, 3400 Spruce Street, Philadelphia, Pennsylvania 19104.

Six patients, three of each type, were further studied as follows: quantitative bacterial cultures of the anterior nares, complete blood count, fasting blood sugar, blood urea nitrogen, serum protein electrophoresis and quantitative immunoglobulins, blood, urine and throat cultures.

RESULTS

In 4 years, fifty cases of Gram-negative folliculitis were identified out of 1200 new cases. The results are summarized in Tables 1 and 2.

TABLE 1. Summary of fifty patients with Gram-negative folliculitis

	Clinical findings	Culture of anterior nares and lesions
Type I Thirty-five cases	Significant, follicular pustules often grouped about the nose (Fig. 1)	Lactose fermenting Gram-negative rods often *Klebsiella–Enterobacter*
Type II Fifteen cases	Deep, nodular and cystic lesions (Fig. 2)	*Proteus*

TABLE 2. Quantitative nasal cultures

Clinical presentation	Gram-negative organism in nose and pustule or cyst	Total aerobic count with %Gram-negative	
		R. nasal flora	L. nasal flora
1. Predominantly pustular eruption right side worse than left	Lactose fermenting Gram-negative rod (LFGNR)	2×10^6(12%)	1×10^6(0·8%)
2. Papular and pustular lesions	LFGNR	7×10^6(90%)	5×10^6(8%)
3. Pustules around nose and on cheek	*E. Coli*	$1{\cdot}4 \times 10^6$(66 1%)	$9{\cdot}4 \times 10^6$(0·1%)
	Klebsiella–Enterobacter	(0%)	(3%)
	LFGNR	(0·1)	(0)
4. Pustular and papular eruption on cheeks	LFGNR	$1{\cdot}2 \times 10^6$(3%)	$1{\cdot}0 \times 10^6$(0 1%)
5. Deep cystic lesions on cheeks, around the nose and on the neck	*Proteus*	2×10^6(4%)	$1{\cdot}5 \times 10^6$(4%)
6. Cystic lesions on cheeks and around nose and pustules about the nose and on chin	*Proteus*	6×10^5(37%)	$7{\cdot}5 \times 10^5$(33%)
	LFGNR	(40%)	(65%)

We now recognize two clinical varieties. Type I is the more common (thirty-five out of fifty) and presents as superficial pustules usually grouped about the nose (Fig. 1). The Gram-negative organism

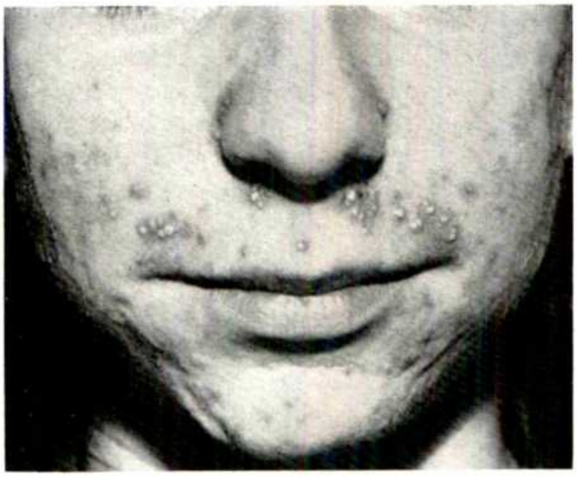

FIGURE 1. Type I Gram-negative folliculitis with grouped follicular pustules centred about the nose.

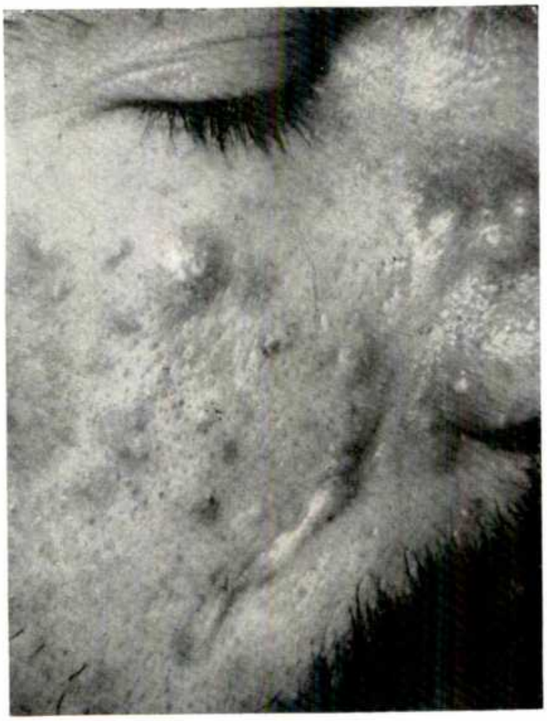

FIGURE 2. Type II with deep indolent abscess lesions. *Proteus* was recovered from these nodular lesions.

is a lactose fermenting rod, which as a rule has not been further identified but in seven patients the following organisms were recovered; *Klebsiella* species in three patients, *Enterobacter* species in four. It should be noted that the lesions regularly contained the more typical acne flora: coagulase negative cocci and *Corynebacterium acnes*. The three patients investigated intensively showed no abnormality in any of the tests and in particular the organism was not cultured from blood, urine or throat.

Deep nodular and cystic lesions are characteristic of Type II (Fig. 2). In this variety *Proteus* are recovered from the anterior nares and the cystic lesions as well as coagulase negative cocci, lipophilic diphtheroids and *C. acnes*.

Typical case history of Type I

R.M. was a 19-year-old male who had been treated for acne vulgaris for 6 years. During that time he received one antibiotic or another including tetracycline, demeclocycline, erythromycin, penicillin, clindamycin and lincomycin. He washed his face exclusively with soaps containing hexachlorophene. Several antibiotics, notably erythromycin and penicillin, seemed to aggravate the eruption. Stopping any helpful antibiotic was followed by rapid exacerbation. Nasal cultures revealed 9,473,000 organisms in the left nose and 1,470,000 in the right with 12% of the left anterior nares flora composed of a lactose fermenting Gram-negative rod and 87% of the right anterior nares flora consisting of the same organism. The organism was sensitive to ampicillin and the patient was placed on 250 mg four times daily. Within 10 days there was a marked improvement. At this point the ampicillin was gradually lowered to 250 mg a day. This dose plus topical application of gentamicin cream to the anterior nares has controlled the pustular lesions. Any attempt to discontinue this regimen results in reappearance of numerous pustules within 3–5 days.

Typical case history of Type II

A.C. was a 28-year-old woman who had been treated for 'acne' since the age of 16. During that time she had been on numerous antibiotics including tetracycline, erythromycin and lincomycin. She was referred to the Acne Clinic for treatment following a sudden explosive onset of deep nodular cystic lesions on her face 4–6 days after having been placed on demeclocycline 150 mg thrice daily. Anterior nares cultures showed 163,000 organisms on the left and 174,000 organisms on the right with both sides having 4% *Proteus mirabilis*. This organism was sensitive to ampicillin. After 2 weeks of ampicillin 250 mg four times daily her face had cleared. At present she is maintained on ampicillin 250 mg twice daily and any attempt to discontinue this drug is followed by the rapid appearance of new cystic lesions.

Effect of antibiotics on the course of Gram-negative folliculitis

Of the series, six patients were inadvertantly placed on an antibiotic to which their Gram-negative organism was resistant. This occurred because culture results were not available when the decision to start antibiotic therapy was made. The antibiotic was demeclocycline 150 mg twice daily in three patients, erythromycin 250 mg four times daily in two and cephalothin 150 mg twice daily in the other. Clinical exacerbation consisting of new pustules and nodular lesions occurred within 5 days. Cultures of new inflammatory lesions revealed the same organism found prior to this course of treatment. Subsequent antibiotic therapy directed against the Gram-negatives resulted in rapid clinical improvement with resolution of pustules and nodular lesions occurring in 7–10 days.

Typical example. B.M. was a 17-year-old white male who had mainly comedo type acne with scattered papules. He had been treated with various antibiotics for 1 year.

The right anterior nares had 500,000 aerobic organisms—2% were *Proteus mirabilis*, the left had

600,000 organisms—3% were *Proteus*. Ten open and ten closed comedones were individually cultured —none of the open comedones had *Proteus* but three out of the five closed comedones had *Proteus* as the predominant aerobic organism.

The strain of *Proteus* was resistant to demeclocycline and sensitive to ampicillin. After 4 days of demeclocycline 300 mg twice daily several large tender cystic lesions appeared on both cheeks. Three cystic lesions revealed a heavy growth of *Proteus*. Subsequent treatment with ampicillin 250 mg four times daily for 2 weeks produced a complete resolution of this flare.

DISCUSSION

Several features of this syndrome have become clearer since our original report. The history is very helpful in suggesting the diagnosis. The response to a course of antibiotics is of particular importance. Patients report exacerbations a few days following institution of certain antibiotics and equally rapid improvement following therapy with other antibiotics. Likewise, relapse following cessation of an effective antibiotic was also reported to occur within a few days. Each of these patient observations could be confirmed during monitored therapy. These findings are distinctly different from acne vulgaris and are more suggestive of a direct bacterial infection. Therapy with an effective antibiotic produces gradual improvement over a period of 4–6 weeks in acne patients. Conversely, relapse is slow following antibiotic withdrawal. In Gram-negative folliculitis, the history of rapid exacerbation results from overgrowth of an organism resistant to the antibiotic. The rapidity of response to antibiotic therapy directed against the Gram-negative indicates suppression of the inciting organism. Relapse following antibiotic withdrawal shows that the unwanted colonist has not been totally eliminated. The reservoir for further attacks of folliculitis appeared to be the nasal mucosa.

It is now more apparent that there are two distinct varieties of this syndrome; the superficial pustular type and the deep nodular variety. In confirming the diagnosis, special care must be exercised in culturing. Gram-negative organisms are sensitive to desiccation, therefore, samples must be taken quickly and cultured as soon as possible. The pustule to be sampled should also be fresh; preferably a small pustule on an erythematous base. Gram-negative organisms are not recovered from every pustule probably because phagocytosis is so effective in older lesions. The nodulo-cystic lesions of Type II, on the other hand, yield *Proteus* regularly.

A characteristic feature of our patients was the long term use of tetracyclines. Strains of Gram-positive and Gram-negative organisms resistant to tetracycline are not uncommon. In a previous study we showed that the continued use of antibiotics leads to a higher rate of colonization of the anterior nares of acne patients by Gram-negative organisms (Marples *et al.*, 1969). In fact, the incidence rate was 73%. However, the Gram-negative organisms represented less than 1% of the total aerobic nasal flora. In patients with Gram-negative folliculitis, the Gram-negative organism usually amounted to more than 3–4% of the total aerobic nasal flora (Table 2). Such an increase might be very significant when one is dealing with a motile virulent organism such as *Proteus*. The higher number of organisms increases the chance of spill over onto the face.

The intensive use of soaps containing hexachlorophene may be another contributing factor. Hexachlorophene is bacteriostatic against Gram-positive organisms and has been associated with overgrowth of Gram-negative bacteria in several situations (Forfar, Gould & MacCabe, 1968; Stetler *et al.*, 1970). The combined action of prolonged use of antibiotics and antibacterial soaps which suppress the Gram-positives creates suitable conditions for proliferation of Gram-negative organisms.

Our treatment regimen is as follows: systemic antibiotics form the first line of attack with the choice dictated by antibiotic sensitivities. Ampicillin will usually be the drug of choice. A daily dose of

1 g is employed for 7–14 days till the folliculitis has cleared. At this point the dose is lowered to a maintenance level. The use of topical antibiotic creams such as 1% chloramphenicol to the anterior nares has seemed helpful in achieving a lower maintainance dose of systemic antibiotic. Multiple antibiotics have the advantage of decreasing the development of antibiotic resistance by Gram-negative bacteria. It should be stressed that many patients have ordinary acne vulgaris in addition to Gram-negative folliculitis. Once the folliculitis has responded, the residual acne must then be treated by other means, e.g. retinoic acid, cryotherapy, etc. To date, we have been unable to 'cure' any of our patients, i.e. some sort of topical and/or systemic antibiotic therapy must be employed to suppress the Gram-negatives. In our previous report we also indicated our failure to establish a normal nasal flora by implantation of non-pathogens into the anterior nares. Several patients have avoided antibiotics altogether for prolonged periods of time (up to 3 years) but without benefit. Fortunately, this superinfection appears to be limited to the skin; no evidence for systemic Gram-negative infections has been found in any of our patients.

We would also emphasize that this syndrome is relatively uncommon and the general benefit from antibiotics in acne vulgaris far outweighs the occasional complication of Gram-negative folliculitis.

ACKNOWLEDGMENTS

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The invasion of burned skin by *Pseudomonas aeruginosa*

SHIRLEY A.CARNEY, R.E.DYSTER AND R.J.JONES

M.R.C. Industrial Injuries and Burns Unit, Birmingham Accident Hospital, Bath Row, Birmingham B15 1NA

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SUMMARY

When standard skin burns of mice were infected with a virulent strain of *Pseudomonas aeruginosa* there was rapid and extensive penetration of the burn and underlying tissues by the organisms. Under similar experimental conditions an avirulent strain of *Ps. aeruginosa* remained localized at the surface of the burned skin.

In vitro experiments showed that heating did not make the proteins of skin more susceptible to hydrolysis by enzymes, since heated and unheated skin were hydrolysed to approximately the same extent by proteinases from both virulent and avirulent strains of *Ps. aeruginosa*.

Burns of the skin are readily colonized by *Ps. aeruginosa* (Jackson, Lowbury & Topley, 1951; Markley *et al.*, 1957; Tumbusch *et al.*, 1961). In patients not receiving effective treatment bacteria can penetrate the burns and underlying tissues and may cause fatal septicaemia (Cason & Lowbury, 1968).

In the present study the bacterial invasion of burned skin was followed by histological and immunofluorescent techniques, using two strains of *Ps. aeruginosa*, P14 (lethal for burned mice, Jones & Lowbury, 1966) and P2AB (not lethal for burned mice). These strains produced toxins of similar lethality, but P14 produced extracellular proteinases more rapidly than P2AB (Carney & Jones, 1968). In the present investigation normal and heat-damaged guinea-pig skin was compared in hydrolysis experiments using extracellular proteinases of *Ps. aeruginosa* P14 and P2AB.

METHODS

Bacteria

Ps. aeruginosa, strains P14 and P2AB, were obtained from the National Collection of Type Cultures and were maintained by weekly subculture on nutrient agar containing horse blood. P14 was highly invasive for burned mice (Jones, 1970, 1971), and produced large amounts of enzymes *in vitro* (Carney & Jones, 1968); P2AB, in the same series of experiments, did not cause lethal invasive infection of burned mice, and produced small amounts of extracellular enzymes.

Burning and infection

Male albino Schofield mice (25g) were given full skin-thickness burns of approximately 5% of the body surface, using methods described by Jones & Lawrence (1964). Burns were made on skin of even

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thickness by ensuring that the skin that was burned was in the anagen phase of the hair growth cycle (Zawacki & Jones, 1967). The surfaces of burns were infected with *Ps. aeruginosa* strain P14 or strain P2AB by spreading 0·1 ml of a saline suspension containing 7×10^8 organisms over the surface of the burn (Jones, Jackson & Lowbury, 1966). After burning and infection mice were kept in individual cages and given food and water *ad lib.*

Histology

On each of 7 days after burning and infection, a piece of skin 2·5 × 0·5 cm consisting of equal parts of burned and unburned skin, was excised from a mouse, killed immediately before excision by ether anaesthesia. Skin was fixed in 95% ethanol at 4°C and processed by the method of Sainte-Marie (1962).

Staining

Sections (5 μm) were stained for 3 min with Harris's haematoxylin, then washed in tap water, differentiated in 0·1% acid/alcohol, blued in tap water, stained for 3 min in 1·0% (w/v) eosin in 0·1% (w/v) calcium nitrate, washed, dehydrated, cleared and mounted in DPX.

Immunofluorescence

Indirect staining technique was used (Holborrow & Johnson, 1967). Antibacterial sera were made using techniques described by Jones (1968). Difco, fluorescein isothiocyanate conjugated goat anti-rabbit globulin, mouse tissue powder, phosphate buffer, and glycerin mountant were used. Photomicrographs were taken on a Leitz Orthomat automatic camera, using Ilford Microneg Pan film for black and white negatives and Kodak materials for the colour photographs.

Enzyme preparations

Material with proteolytic activity was prepared from culture filtrates of *Ps. aeruginosa* grown in a medium described by Morihara (1963) for 3 days at 37°C. The culture filtrates were sterilized by centrifugation (10,000 rev/min for 30 min at 4°C) and positive pressure filtration (GS millipore membrane, pore size 0·22 μm.). Phosphate was precipitated from the culture filtrate by the addition of saturated $CaCl_2$ solution. The precipitate was removed by filtration through Hyflo super-cel. Solid $(NH_4)_2SO_4$ was added to the filtrate at the rate of 361 g/l (60% w/v of the amount required to saturate the solution) and the solution allowed to stand for 18 h at 4°C. The protein precipitate which formed was removed by filtration and redissolved in 10 ml 0·01 M $CaCl_2$ solution, and remaining insoluble material mostly $CaSO_4$, was discarded. The soluble protein solution was dialysed against 1 litre 0·002 M $CaCl_2$ solution for 18 h at 4°C then twice against 1 litre of distilled water for 18 h at 4°C and finally freeze-dried. Fractionation of this material on G-200 Sephadex showed that the principal proteolytic component had a molecular weight of 47,000 (Carney, 1971).

Hydrolysis of skin proteins by Ps. aeruginosa *proteinase preparations*

The hydrolytic activities of the P14 and P2AB enzyme preparations were tested on burned and unburned guinea-pig ear skin. On the day prior to the experiment the ears of a Portland guinea-pig were shaved and disinfected with 2% iodine in 70% ethanol and Mycil cream (BDH). Heated skin (provided by Dr J. C. Lawrence) was obtained by placing one ear of the anaesthetized guinea-pig in water heated at 40°C for 30 min. These were the minimum conditions found to cause irreversible heat damage as shown by skin respirometry (Lawrence, 1969). The other ear of the same animal provided unheated skin for control experiments. After the ear had been heated for 30 min the guinea-pig was killed by ether anaesthesia, and pieces of skin, having equal amounts of dermis and epidermis were cut from the dorsal surfaces of the ears with a scalpel (Cruickshank, 1954). Each piece of skin was weighed and then floated on 0·1 ml of sterile saline, or 0·1 ml of enzyme solution (10 mg/ml in sterile saline) in a sterile 3 × 0·5 in stoppered tube. Tubes containing 0·1 ml volumes of enzyme solution without skin were also included in the experiment. All tubes were incubated for 24 h at 37°C.

Estimation of amino acids released by hydrolysis

After incubation the free amino acids released into the medium were estimated. The solutions on which the pieces of skin had been incubated were filtered free from skin residues. The skin residues were washed in the filters three times with distilled water and the washings added to the filtrates already collected. These solutions were freeze-dried and the freeze-dried material redissolved in 0·5 ml of 1·0 % (w/v) aqueous ninhydrin solution. Ninhydrin colours were generated by heating the solution in a boiling water bath for 10 min and read after cooling at $\lambda = 570$ nm in a Unicam SP500 spectrophotometer.

The amount of free amino acids released into the incubation medium from the skin was expressed as mg of glycine. For this purpose a standard curve for glycine was prepared using the ninhydrin method, and used to convert the ninhydrin values obtained experimentally to mg of glycine. These glycine values were expressed as mg of protein hydrolysed ('polyglycine') and related to the fresh weights of the original skin samples.

RESULTS

Infection of experimental skin burns with virulent (P14) and avirulent (P2AB) strains of Ps. aeruginosa

Burned mice were divided into two groups. The first group was infected with a virulent strain of *Ps. aeruginosa* (P14), the second group was infected with the same number of organisms of an avirulent strain of *Ps. aeruginosa* (P2AB). Specimens of burned, infected skin were excised from a different mouse from each group, every day for 7 days after burning and infection.

Figure 1 shows a transverse section of mouse skin 1 day after burning and infection with P14. Hair follicles which are a predominant feature of mouse skin can be seen in the dermis and in the fat layer above the panniculus carnosus. The mottled appearance of the dermis is due to oedema and heat coagulation caused by burning.

During the 24 h following inoculation of the burn with P14 there was a rapid multiplication of bacteria both on the surface of the burn and in hair follicles. P2AB showed little evidence of either proliferation on the surface of the burn or penetration of the hair follicles.

Fluorescent micrographs (Plate 1a and b) of burned mouse skin 1 day after infection with P14, show the extent of bacterial multiplication and invasion. P14 was found not only in hair follicles but also in adjacent dermis (Plate 1a); the bacteria had spread through hypodermal tissues to the panniculus carnosus where there was evidence of proliferaton in and around the adventitia of an artery (Plate 1b). Two days after burning, mice infected with P2AB showed no extensive bacterial invasion or multiplication on the surface of the burn.

The leukocyte response in mice infected for 1 or 2 days with P14 was more generalized than that of mice infected with P2AB. Compared with the well developed inflammatory response—the dark staining zone at the interface of the burned and unburned tissue, shown in Plate 2—the leukocyte response in the burns of mice 1 and 2 days after infection with P14 was scant, disorganized and scattered thinly throughout both burned and unburned tissue.

Between 3 and 4 days after burned mice had been infected with P14, the burn showed evidence of gross disintegrative necrosis accompanied by bacterial invasion of the adjacent unburned tissues. The burns of mice infected with P2AB were not necrotic and as early as 4 days after burning and infection with P2AB it was found (Plate 3) that new epithelial cells were forcing up the edges of the burn slough and initiating the healing processes of slough rejection and re-epithelialization. In contrast with Plate 3, Plate 4 shows the extensive necrosis in the burned slough and adjacent marginal tissue of mice infected for 3 days with *Ps. aeruginosa* P14. The advance of new epithelial cells has stopped at the necrotic tissue beneath the granulation layer at the margin of the burn.

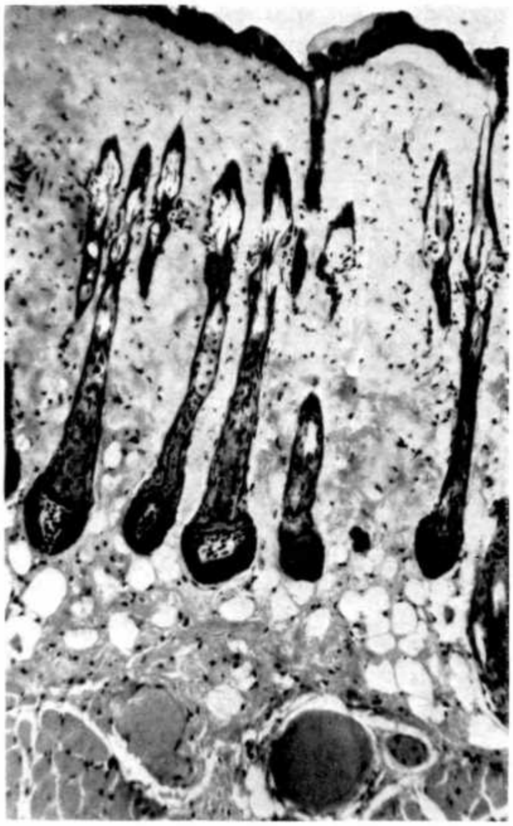
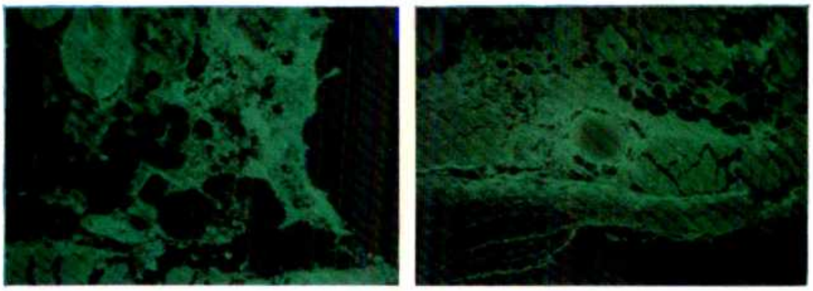


FIGURE 1. Mouse skin 24 h after burning and infection with *Pseudomonas aeruginosa*. Epithelium, dermis with characteristic hair follicles, stratum adiposum and panniculus carnosus are clearly defined. The mottled appearance of the dermis is due to oedema and coagulation of the dermis by heat (H & E × 150).

Plate 5 is a fluorescent micrograph of the burned slough of a mouse infected for 4 days with P2AB; it illustrates the limited extent of invasion by P2AB. The bacteria have multiplied in the outer layers of the slough but have remained localized and have hardly penetrated the dermis. If Plate 5 is compared with Plate 1a and b, which are fluorescent micrographs showing the progressive invasion by P14 occuring 1 day after infection, the marked differences between the invasiveness for skin burns of P14 and P2AB can be clearly seen. Invasiveness of P14 was characterized by rapid multiplication on the surface of the burn and in the hair follicles, together with spread of the bacteria into the slough and underlying tissues within 48 h of the initial colonisation; whereas P2AB remained localized in the outer regions of the burn and failed to penetrate the granulation layer which formed beneath the slough.

1A



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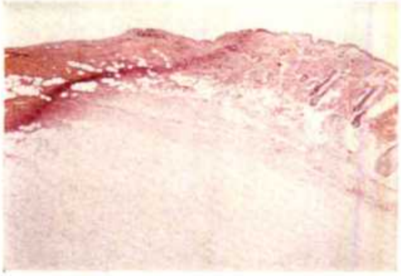

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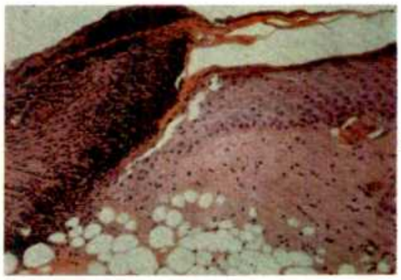

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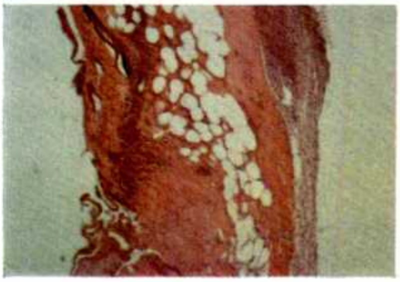

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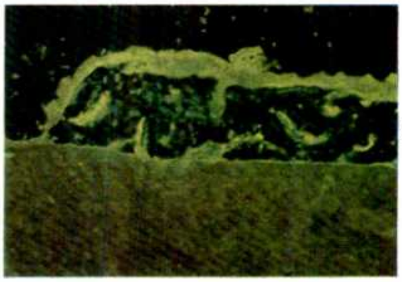

PLATE 1. Mouse skin 24 h after burning and infection with virulent *Ps. aeruginosa*, P14 showing: (a) *Pseudomonas* in bulb of hair follicle (upper LHS) in the dermis and in the hypodermal tissue above the panniculus carnosus (fluorescent antibody stain × 540). (b) proliferation of *Pseudomonas* in and around the adventitia of an artery in the panniculus carnosus (fluorescent antibody stain × 225).

PLATE 2. Mouse skin 1 day after burning and infection with an avirulent strain of *Ps. aeruginosa*, P2 AB, showing a well developed leukocytic response (dark zone) at the interface of burn slough and unburned tissue (H & E × 150).

PLATE 3. Mouse skin 4 days after burning and infection with avirulent *Ps. aeruginosa*, P2AB. The unburned epidermis adjoining the slough was several cells thick and the slough was beginning to separate at the interface. Epithelial cells were beginning to migrate beneath the granulation layer, forcing up the edges of the slough (H & E × 225).

PLATE 4. Mouse skin 3 days after burning and infection with *Ps. aeruginosa* P14, showing necrotic slough. Bacteria have penetrated the granulation layer and were found in adjacent unburned skin. There is no evidence of slough rejection by the epidermis (H & E × 225).

PLATE 5. Mouse skin 4 days after burning and infection with avirulent *Ps. aeruginosa*, P2AB, showing bacteria mainly confined to the outer regions of the slough and excluded from the dermis (fluorescent antibody stain × 225).

Hydrolysis of skin proteins by Ps. aeruginosa *proteinase preparations*

Residues from enzyme solutions which had been incubated without skin produced no colour with ninhydrin, showing that the enzyme preparation did not autolyse. Unheated guinea-pig ear skin incubated for 24 h at 37°C in the presence of 10 mg/ml P14 proteinase lost three times as much protein as control samples (Table 1). The effect of incubation with the same enzyme was even more marked when heated skin was used. However, the unheated skin samples incubated without enzyme lost more protein (see Tables 1 and 2) than did the heated skin samples, presumably as a result of autolysis.

TABLE 1. The effect of Ps. aeruginosa P14 proteinases on guinea-pig ear skin incubated for 24 h at 37°C

Incubation medium	No. of samples	% wt loss (mg protein hydrolysed/100 mg fresh wt of skin)	Standard error of mean	Significance (by *t* test)
Unheated skin				
Saline only	3	2·07	0·0080	
Saline +P14 Enzymes	3	6·01	0·0040	$P<0·05$
Heated skin				
Saline only	3	0·63	0·0042	
Saline +P14 Enzymes	3	3·47	0·0083	$P<0·01$

TABLE 2. Effect of Ps. aeruginosa P2AB proteinases on guinea-pig ear skin incubated for 24 h at 37°C

Incubation medium	No. of samples	% wt loss (mg protein hydrolysed/100 mg fresh wt of skin)	Standard error of mean	Significance (by *t* test)
Unheated skin				
Saline only	3	1·66	0·0018	
Saline + P2AB Enzymes	2	3·32	0·0013	$P<0·02$
Heated skin				
Saline only	3	0·98	0·0022	
Saline + P2AB Enzymes	3	2·22	0·0011	$P<0·01$

In a similar experiment P2AB proteinase caused a significant increase in protein loss from heated and unheated guinea-pig skin (Table 2). An approximately two-fold increase in protein loss was observed in each case. In this experiment also unheated skin samples lost more protein during incubation than did the heated samples.

DISCUSSION

The histological studies described here demonstrated that the progress of invasion of burned skin by a virulent strain of *Ps. aeruginosa* (P14) differed considerably from that of an avirulent strain (P2AB). After inoculation of burned skin, the initial stages (first 48 h) of invasion by P14 were characterized by an intense bacterial proliferation on the surface of the burn and in the hair follicles. This was followed by infiltration and multiplication in the hypodermis, and spread into the dorsal muscles. In contrast, under similar experimental conditions strain P2AB failed to penetrate the dermis, remained localized in the outer regions of the burned skin, and immunofluorescence showed there was little evidence of proliferation.

The *in vitro* experiments did not entirely confirm the view that heat damage renders skin proteins more susceptible to attack by bacterial proteinases. However, the release of amino acids from unheated as well as from heated skin in the absence of bacterial proteinase suggests that autolytic enzymes were contibuting to the hydrolysis. It is likely that in heat-damaged skin there is both partial inactivation of autolytic enzymes, counteracted by partial denaturation of proteins rendering them more liable to attack by the autolytic activity remaining, as some degree of denaturation would be necessary before collagen could be attacked by non-specific bacterial proteinases. This appears to be the most likely explanation in the light of the difference in invasive properties of *Ps. aeruginosa* strains P14 and P2AB demonstrated histologically in the present paper, and their known difference in ability to produce extracellular proteinases (Carney & Jones, 1968), but further work is required to elucidate the mechanism of the invasion process.

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Hæmophilus influenzæ cellulitis

CASE PRESENTATION AND REVIEW OF THE LITERATURE

JAMES E. RASMUSSEN

Department of Dermatology, University of Oregon Medical School, Portland, Oregon 97201, U.S.A.

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SUMMARY

All reported cases of *Hæmophilus influenzæ* cellulitis are reviewed and compared. A typical patient is a male between the ages of 1–3 years. He is acutely ill with a red-blue cellulitis of the face. Swelling of the involved area is massive and rapid in onset. Both temperature and the number of leukocytes are markedly elevated. *Hæmophilus influenzæ* type B can usually be isolated from cultures of the blood or nasopharynx. Appropriate antibiotic therapy produces prompt remission of both signs and symptoms.

The first report of cellulitis due to *Hæmophilus influenzæ* appeared in 1953 (Alexander). Cases described in 1957 (Green & Fousek) and 1959 (Thilenius & Carter) further delineated this condition. In 1965, Feingold & Gellis reported nine additional cases and summarized those previously mentioned. They portrayed a typical patient as a child 6–24 months of age, acutely ill with high fever, leukocytosis, a red-blue cellulitis of the face and blood cultures positive for *Hæmophilus influenzæ* type B. Since 1965, only two additional primary articles on this subject have appeared (Medina & Fine, 1967; Feigin, Shackelford & Keeney, 1971).

The purpose of this paper is to describe and illustrate another example of *Hæmophilus influenzæ* cellulitis with the intention of renewing the vivid image of this process. In addition, details of all previously reported cases are presented for review (Table 1).

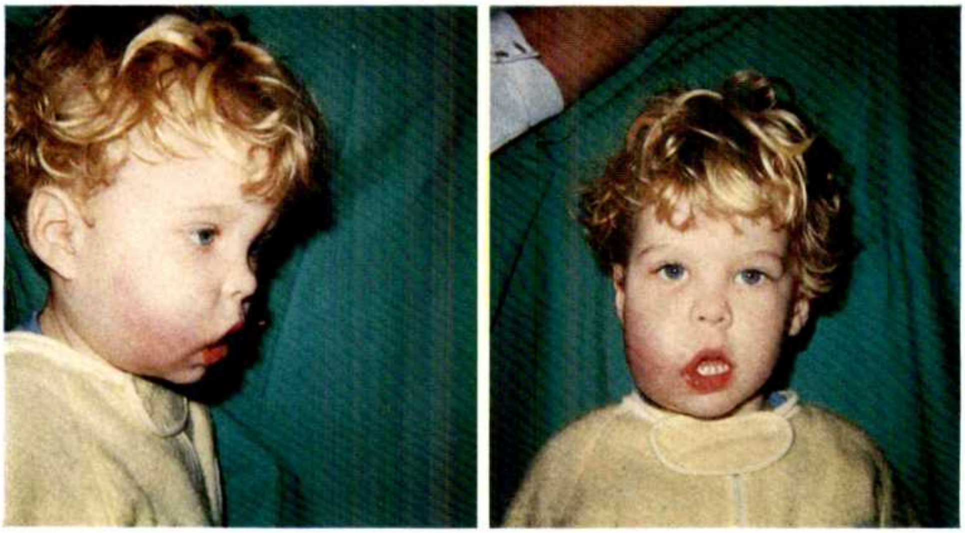
CASE REPORT

E.J.R., 34-month-old son of the author, had been in excellent health until 24 March 1972 when he developed photophobia, malaise, a rectal temperature of 39.8°C and a cloudy left tympanic membrane. His condition remained unchanged until the next evening when he complained of discomfort in his right cheek. He awoke 4 h later with pain and massive œdema in this area. He was moderately ill with a rectal temperature of 40.7°C. An 8 × 6 cm tender, irregularly defined area of firm swelling involved the entire right cheek and the angle of the mouth. The borders were erythematous while the central portion was blue-red (Plates 1 and 2). The buccal mucosa on the inner aspect of the right cheek was pale and œdematous. Apart from his general toxicity, the only other pertinent finding on physical examination was a dull red, left tympanic membrane. There was no dental pathology. Both ethmoid cavities were minimally clouded on roentgenogram. The white blood cell count was 14,700 with 74% polymorphonuclear leukocytes.

TABLE 1. Review of cases

Author and patient no.	Age (months)	Sex	Site of cellulitis	Colour	Otitis or sinusitis	Temp. (rectal)	WBC	%PMN	Site of isolation of *H. influenzae*		Course and sequelae
									Blood	Nasopharynx	
Alexander Case 1	—	—	Submental and anterior cervical	—	—	—	—	—	—	—	Meningitis
Green *et al.*											
Case 2	24	M	L. cheek	Dusky blue-red	No	40·4	25,000	69	+, Type B		Cleared in 2 days
Case 3	9	M	R. cheek, R. knee	Slightly red	No	40·0	26,000	64	+, Type B		Afebrile in 7 days
Case 4	12	F	R. cheek	Reddish purple	No	39·6	24,600	77	NG	+, Type B	Afebrile in 1 day
Case 5	15	F	L. cheek	Purple	No	40·7	32,000	66	NG	+, Type B	No sequelae
Case 6	21	M	R. periorbital	Red	Both tympanic membranes red	39·8	29,000	84	+, Type B		Swelling resolved in 3 days
Case 7	12	M	L. arm	No colour given	No	38·4	20,000	64	+, Type B	+, No type	Resolved in 2 days
Thulenius & Carter Case 8	6	M	L. leg	Bright red	No	40·4	22,450	70	+, Type B		Slow resolution in 2 weeks
Feingold & Carter											
Case 9	10		L. arm	Bluish red	—	39·4	—	—	+, Type B		—
Case 10	8		R. upper	Purplish red	—	40·0	—	—	+, Type B		—
Case 11	8		R. cheek	Red	—	40·6	—	—	+, Type B		—
Case 12	7		R. periorbital	Red	—	40·0	—	—	+, Type B		—
Case 13	9	F	L. cheek	Purplish red	—	39·6	—	—	+, Type B	+, No type	—
Case 14	8		L. cheek	Bluish red	—	40·0	—	—	+, Type B		—
Case 15	18		R. cheek	Bluish red	—	38·8	—	—	No *H. influenzae* found		—
Case 16	17		R. cheek	Red	—	40·6	—	—	NG	+, No type	—
Case 17	21		L. hand	Red	—	40·6	—	—	+, Type B	+, No type	—
Medina & Fine Case 18	8	M	R. periorbital	Sturge-Weber Synd. R. side of face	No	40·0	28,400	—	+, Type B	+, Type B	Afebrile in 12 h
Case 19	36	M	R. periorbital	Purple	X-ray evidence of clouding of both maxillary sinuses	38·3	27,000	—	+, Type B		Afebrile in 12 h
Case 20	19	M	R. eyelid, cheek, forehead	Purple	Maxillary and ethmoid sinuses hazy bilaterally on X-ray	39·2	20,800	—	+, Type B	+, Type B	Afebrile in 36 h
Fine Cases 21–23		M	Periorbital area	—	—	—	—	—	—	—	—
		M	Periorbital area	—	—	—	—	—	—	—	—
		M	Periorbital area	—	—	—	—	—	—	—	—
Case 24		F	Periorbital area	—	—	—	—	—	—	—	—
Felgin *et al.* Case 25	2	F	L. hand	Red	Both tympanic membranes inflamed	39·0	25,733	60	+, Type B		Afebrile in 24 h abcess, left palmar space
Case 26 (present case)	36	M	R. cheek	Red-blue	Ethmoid sinuses clouded on X-ray, left tympanic membrane red	40·0	14,700	70	+, No type		Afebrile in 36 h

NG, no growth; —, specific data not given in report.



PLATES 1 and 2. Anterior and lateral views of the patient taken 12 h after the onset of the swelling demonstrating the massive œdema and peculiar colour.

The initial diagnosis was otitis, sinusitis and cellulitis. Procaine penicillin was given intramuscularly plus an oral suspension of penicillin V (125 mg/5 ml) and 'triple sulfa' (each 5 ml contained 0·166 g each of sulphadiazine, sulphamerazine and sulphamethazine).

He improved considerably within 36 h and was normothermic at that time. The swelling of his cheek resolved slowly in 1 week and the colour progressed from the red-blue seen initially to a shade of yellow-green resembling a bruise. *Hæmophilus influenzæ* of unknown type was isolated from a blood culture after 5 days' incubation.

DISCUSSION

The most complete description of *Hæmophilus influenzæ* cellulitis is contained in *Dermatology in General Medicine* (Weinberg & Swartz, 1971). This syndrome has not been reported in the European literature, and standard paediatric texts make little or no reference to this problem. With the addition of the present patient, the number of cases reported in the medical literature is twenty-six. They have been unequally divided among six previous articles, none of which includes a photograph of an affected patient. The three patients described by Tomic-Karovic (1953) and previously cited (Thilenius & Carter, 1959) as examples of this syndrome have not been included in this review. Tomic-Karavic isolated the organism from a 2-year-old draining abscess in a 7-year-old boy and from 'urethral glands' in two other adults. Other than infection with *Hæmophilus influenzæ* his patients showed none of the features of *Hæmophilus influenzæ* cellulitis.

Age

Most patients have been between 6 and 36 months of age. The recent report of a 2-month-old patient (Feigin *et al.*, 1971) demonstrates that children younger than 6 months are also susceptible. Even younger patients might possibly be seen, since Graber *et al.* (1971) demonstrated that only 10% of newborns had antibody bactericidal to type B *Hæmophilus influenzæ*.

Sex

73% (thirteen out of eighteen) of the patients for whom data are available have been males (Fine, personal communication). This is not statistically significant and may be fortuitous.

Prodromal symptoms

These consist of 1–2 days of nonspecific cough, pharyngitis, fever and malaise.

Presenting manifestations

The child is moderately to severely ill with a rectal temperature of 39–41 °C. There is rapid onset of swelling of the face, less commonly of the upper extremities and rarely of the lower extremities. The explosiveness of this process may be diagnostically significant since some authors (Feigin *et al.*, 1971) suggest that facial cellulitis due to other causes generally evolves over several days. Nearly all patients with *Hæmophilus* cellulitis sought medical attention within 24 h of the onset of the swelling.

Site of involvement

There is massive unilateral swelling of the cheek or periorbital area. Other sites of involvement have included the neck (Alexander, 1953), the right leg (Green & Fousek 1957; Thilenius & Carter, 1959), the left arm (Green & Fousek, 1957; Feingold & Gellis, 1965) and the left hand (Feingold & Gellis, 1965; Feigin *et al.*, 1971). Only one patient had involvement of two sites (Green & Fousek, 1957).

The colour of the lesion

This ranges from red to blue-red to purple. As the process resolves, it may resemble a week-old bruise. The specific reason for the peculiar coloration is unknown. *Haemophilus influenzae* is not haemolytic *in vitro*.

Associated findings

A number of these patients also have otitis or ethmoid sinusitis which may have been the source of the septicaemia and cellulitis, but the exact mechanisms of infection are not known.

Laboratory findings

There is a white blood cell count of 15,000–30,000 with an increase in the percentage of polymorphonuclear leukocytes. *Haemophilus influenzae*, usually type B, can be isolated from cultures of the blood and, less often, the throat.

Course and prognosis

In spite of the gross bacteriaemia with *Haemophilus influenzae*, meningitis developed in only one patient (Alexander, 1953). There are no other reported complications. Only once have the lesions been described as suppurative or fluctuant (Feigin *et al.*, 1971). Therapy with the appropriate antibiotic (ampicillin, sulphonamides, chloramphenicol or tetracycline) or a combination of penicillin and streptomycin (Gellis & Kagin, 1971) produces a prompt reduction in both the inflammation and the fever.

Awareness that these signs and symptoms signal the presence of an infection with *Haemophilus influenzae* is the key to effective therapy.

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Malignant melanoma in East Anglia, England

AN 11-YEAR SURVEY BY SITE AND TYPE

L.BAKOS* AND A.L.MACMILLAN

Addenbrooke's Hospital, Cambridge

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SUMMARY

A series of 137 patients with primary malignant melanoma of skin occurring in the Cambridge area in 1961–71 is reviewed. The histological material has been classified into lentigo maligna melanoma, superficial spreading melanoma and nodular melanoma, using the criteria of Clark *et al.* (1969). Consideration of type, site and incidence of melanoma when compared with previously reported series from Boston and Sydney supports the hypothesis that sunlight induces malignant melanoma in unexposed skin.

Having accepted the hypothesis that melanoma is induced by exposure of skin to ultraviolet light, Lee & Merrill (1970), to explain the apparent induction of melanoma by ultraviolet light in unexposed skin, further postulated a hypothetical solar circulating factor that is produced in skin exposed to ultraviolet light and that stimulates malignant change in pigment cells in unexposed skin. The description of induction of melanoma in unexposed skin was based on skin distribution of melanoma in regions of low and high incidence and later on a more satisfactory basis on the comparison of series of melanomas classified similarly from Sydney, Australia, latitude 34°S (McGovern, 1970) and from Boston, U.S.A., latitude 43°N (Clark *et al.*, 1969). To test this hypothesis further, we have examined a series of melanomas occurring in and about Cambridge, England, in the East Anglian region, latitude 52°N.

MATERIAL AND METHODS

All histological material available in Addenbrooke's Hospital, Cambridge, relating to primary malignant melanoma of the skin for the years 1961–71, has been examined. Malignant melanoma was diagnosed when malignant melanocytes were found in the dermis. Cases were traced from the diagnostic indices of the Cancer Registration Bureau and of the departments of dermatology, pathology and radiotherapy. Material relating to 137 patients with malignant melanoma who were resident within the East Anglian region has been classified into three types, as (1) lentigo maligna melanoma, (2) superficial spreading melanoma or (3) nodular melanoma (Clark *et al.*, 1969). These terms are synonymous with (1) melanoma arising in Hutchinson's melanotic freckle, (2) melanoma arising in premalignant melanosis (pagetoid melanoma) and (3) nodular melanoma (McGovern, 1970).

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* Present Address: Division of Dermatology, Department of Internal Medicine, Faculty of Medicine, Federal University of Rio Grande do Sul, Porto Alegre, Brazil.

No difficulty was found in classifying the material histologically. Nodular melanoma shows malignant melanocytes confined to the dermal tumour and appearing in some instances in the epidermis, but then only by direct outwards invasion. Superficial spreading melanoma shows pagetoid invasion of epidermis and often stratum corneum by single cells or theques of atypical melanocytes, usually epithelioid, extending much beyond the site or sites of dermal invasion. A similar pagetoid invasion occurs in some benign melanocytic naevi, particularly from children, but these differ in lacking atypicality of the intra-epidermal melanocytes, particularly of the nuclei. Lentigo maligna melanoma shows atypical pleomorphic often spindle cell melanocytes in the basal layer of the epidermis surrounding the site or sites of dermal invasion.

The site of the melanoma was obtained from case records. As division of skin into different sites varies between series, the site has been arbitrarily classified as on exposed skin (head, neck, forearm and hand excluding palm, and female leg excluding sole) or as on unexposed skin (all other skin sites).

The incidence of melanoma was obtained from the annual reports of the East Anglian Cancer Registration Bureau.

RESULTS

Types of melanoma

Of the 137 melanomas examined fifteen were lentigo maligna melanomas, sixty-nine were superficial spreading melanomas and fifty-three were nodular melanomas. The age distribution is shown in Fig. 1. Almost all lentigo maligna melanomas occurred in skin showing marked solar elastosis. The median age at diagnosis of lentigo maligna melanoma patients was 70–79, of superficial spreading melanoma patients 40–49 years and for nodular melanoma patients there was approximately constant incidence from 30–79 years. Both this Cambridge series and that from Boston suggest that the age incidence of nodular melanoma has a bimodal distribution. This is not supported by the Sydney series. The sex distribution expressed as a ratio of male to female was 1 : 2·5 for all melanomas, 1 : 3

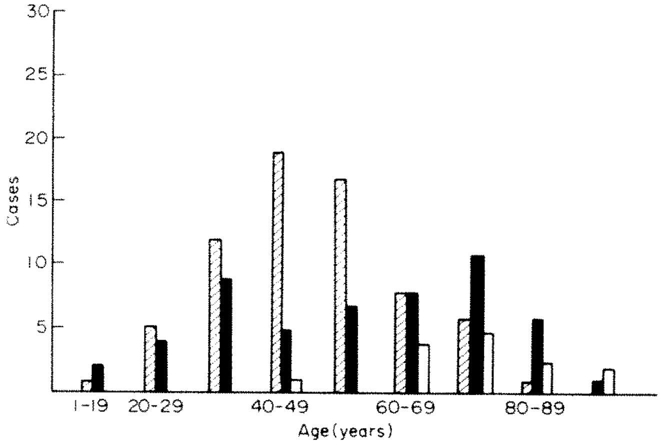

FIGURE 1. Histogram illustrating the age incidence of the three types of melanoma. Diagonally hatched columns, superficial spreading melanoma; closed columns, nodular melanoma; open columns, lentigo maligna melanoma.

TABLE 1. Relative frequency (as a percentage of all melanomas) of each type of melanoma in Cambridge, Boston and Sydney

	Cambridge	Boston	Sydney
Lentigo maligna melanoma	11	14	10
Superficial spreading melanoma	50	55	43
Nodular melanoma	39	32	48

for lentigo maligna melanoma, 1 : 3 for superficial spreading melanoma and 1 : 2 for nodular melanoma.

The relative frequencies of each type of melanoma in this series is compared with the frequencies in the series from Boston and Sydney in Table 1. The relative frequency of each type of melanoma is approximately the same in the three series, 10% lentigo maligna melanoma, 40% nodular melanoma and 50% superficial spreading melanoma. Such variation as occurs does not change consistently with latitude.

Site of melanoma

Site of melanoma is arbitrarily described as on 'exposed skin' (head, neck, forearm and hand excluding palm, and female leg excluding sole) or as on the trunk. This facilitates comparison between series. Occurrence on the trunk is used as an index of occurrence on unexposed skin. Results are compared with those from the Boston and Sydney series in Table 2. Using these arbitrary indices of exposed and unexposed skin, there is no evidence of any consistent change in melanoma distribution between sun-exposed and other skin occurring with change in latitude.

TABLE 2. Relative frequency (as a percentage of all melanomas in the series) of occurrence of melanoma on exposed skin and on the trunk (results from 135 cases, site was not recorded in two cases)

	On exposed skin			On trunk		
	Cambridge	Boston	Sydney	Cambridge	Boston	Sydney
All melanomas	68	49	60	22	25	12
Lentigo maligna melanoma	93*	100	100	0	0	0
Superficial spreading melanoma	69	44	52	24	28	14
Nodular melanoma	60†	36	65	25	30	13

* One lentigo maligna melanoma occurred on the leg of a man aged 41.

† This includes two patients with xeroderma pigmentosum who developed three nodular melanomas of exposed skin.

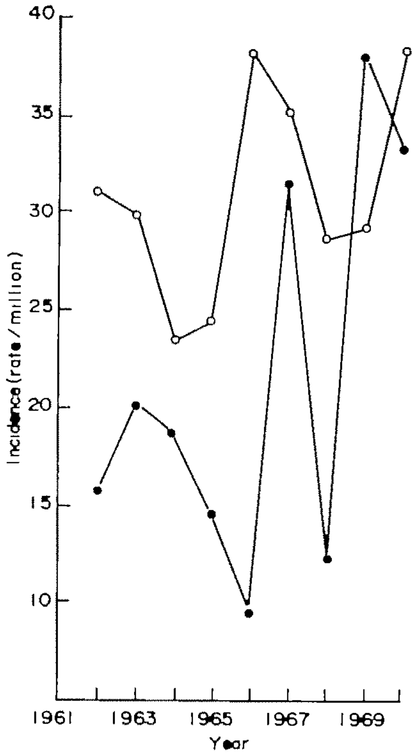

FIGURE 2. Melanoma incidence (cases/million living) for the East Anglian region of England, latitude 52–54° (data from the Annual Reports of the East Anglian Cancer Registration Bureau, Addenbrooke's Hospital, Cambridge). ●, Males; ○, females.

Melanoma incidence

The histological material from melanomas which has been examined came from patients living in the East Anglian region of England. There were no patients with deeply pigmented skin. The annual melanoma incidence for 1961–70 (Fig. 2) has been obtained from the Annual Reports of the East Anglian Cancer Registration Bureau. From available indices this Bureau is highly successful in tracing cases occurring in the region (Registrar General, 1968b). The incidence in neighbouring regions (Doll, Muir & Waterhouse, 1970) and in England and Wales (Registrar General, 1968b) is similar.

TABLE 3. Average melanoma incidence 1963–67, cases/million living in Cambridge and Boston

	Cambridge*	Boston†
Males	18	38
Females	26	36

* Data from Annual Reports of East Anglia Cancer Registration Bureau.

† Data from neighbouring state of Connecticut (Eisenberg & Connelly, 1970)

For comparison with the Boston series, melanoma incidence for 1963–65 is available for the neighbouring state of Connecticut (Eisenberg & Connelly, 1970). These data and the corresponding figures for Cambridge (Table 3) suggest a much higher incidence in Boston than in Cambridge. Incidence figures for Sydney have not been traced. Mortality figures can be used, but are a poor substitute as their accuracy and interpretation are affected by many factors. Lee & Merrill (1971) quote mortality figures for both Sydney and Boston, but do not give the years to which they apply. For England and Wales there has been a gradual increase in melanoma mortality of 50% from 1950 to 1967 (Registrar General, 1967). In Table 4, the mortality figures from Lee & Merrill (1971) are compared with average mortality figures 1967–70 for England and Wales (Registrar General, 1968, 1970, 1971, 1972) showing that melanoma mortality increases as latitude decreases.

TABLE 4. Melanoma mortality/million living (sources of data described in text)

	Sydney	Boston	England and Wales 1967–70
Males	28	21	10
Females	22	16	12

DISCUSSION

The implication of exposure to sunlight as a causative factor in malignant melanoma of skin in populations of European descent is supported by four sets of information. The inverse relationship between latitude of residence and melanoma incidence or mortality has been reported repeatedly (McGovern, 1952; Lancaster, 1956; Lee & Merrill, 1970). Incidence varies from about 20/million in England and Wales to 250/million in coastal regions of Queensland, Australia (Davies, Herron & McLeod, 1966). Two reports agree that within one region there is greater exposure to sunlight from occupation, habit or hobbies in melanoma patients than in a control group (Lancaster & Nelson, 1957; Gellin, Kopf & Garfinkel, 1969). Further, both these reports agree that in the melanoma patients there is an excess of subjects with fair complexion and red hair who sunburn easily and sun tan with difficulty. Finally, in xeroderma pigmentosum, a hereditary skin disorder with greatly increased liability to skin carcinoma of exposed skin, there is an increased incidence of melanoma of exposed skin (Table 2). The incidence and mortality figures quoted for Cambridge, England, for Boston, U.S.A., and for Sydney, Australia, support the hypothesis that in populations of European descent incidence of melanoma of the skin is inversely related to latitude of residence.

Thus, it appears likely that melanomas are induced by exposure to ultraviolet light. Similarly, there is an increase in incidence of squamous cell carcinoma of skin and basal cell carcinoma with increased exposure to ultraviolet light. But on exposed skin of face and ear, while squamous cell carcinomas occur at sites of maximal ultraviolet light irradiation, more than one-third of basal cell carcinomas occur at sites which receive less than 20% of the maximum possible ultraviolet light dose (Urbach, Davies & Forbes, 1966). Melanomas occur in three forms which differ in clinical appearance, distribution between exposed and unexposed skin and prognosis (Clark *et al.*, 1969; McGovern, 1970). Of these three forms, lentigo maligna melanoma is the only one which, like basal cell carcinoma and squamous cell carcinoma of the skin, is found almost exclusively on exposed skin (Wayte & Helwig, 1968; Clark & Mihm, 1969). Both superficial spreading melanoma and nodular melanoma occur on both exposed and unexposed skin, occurring most often on head and neck, and female leg. A direct local effect of ultraviolet light in inducing melanoma would be expected (1) to increase the incidence of melanoma of exposed skin and (2) to increase the relative incidence of lentigo maligna melanoma.

Comparison of the series from Cambridge, Boston and Sydney indicate that there is an increase in incidence of melanomas (Table 3) but that this is not confined to exposed skin (Table 2), nor does it selectively affect lentigo maligna melanoma (Table 1). Such variation in relative frequency of the types of melanoma as is found (Table 1) suggests that increasing melanoma incidence accompanying increasing ultraviolet irradiation does not affect the proportion of lentigo maligna melanoma, but increases the frequency of nodular melanoma at the expense of the superficial spreading type.

Thus, our findings support the hypothesis that sunlight exposure of skin may induce nodular and superficial spreading melanomas on unexposed skin. The concept that irradiation may induce pathological changes at unirradiated sites is novel and its importance has yet to be assessed.

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A histochemical profile of lichen simplex*

R.MARKS AND G.C.WELLS

Department of Pathology, St John's Hospital for Diseases of the Skin,
Lisle Street, Leicester Square, London, W.C.2

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SUMMARY

Enzyme histochemical tests were performed on biopsies taken from patches of lichen simplex in eighteen patients and the results were contrasted with previous findings in psoriasis and lichen planus. The mitochondrial enzyme reactions were found to be increased within the epidermis, differing from those in lichen planus, in which there is a decreased density of reaction product for these tests. The non-specific esterase band was increased in width in lichen simplex contrasting with the absence of a discrete band in psoriasis. The DOPA reaction demonstrated the presence of positively reacting cells in the basal layer, and the ATPase reaction showed that there were many ATPase positive dendritic cells within the epidermis. It appears that in lichen simplex there is hyperplasia of all components of the epidermis in contrast to psoriasis where it seems the hyperplasia is almost restricted to keratinocytes.

Lichen simplex is characterized by epidermal hypertrophy and a high rate of epidermal cell production. In another publication we have contrasted the epidermal cell kinetics in lichen simplex with those of psoriasis on the one hand and lichen planus on the other (Marks & Wells, 1973). We now report histochemical observations in lichen simplex and discuss their significance in relation to the epidermal cell kinetics of this condition and some other dermatoses characterized by epidermal thickening.

PATIENTS AND METHODS

Eighteen patients with typical patches of lichen simplex were studied. There were twelve men and six women, and the mean age of this group was 40·6. The lichenified areas involved the following sites: wrist and forearm (seven), shins or ankles (ten), and the back of neck (one). In addition, for comparison four active lesions from patients with psoriasis and normal skin from five healthy individuals was biopsied. Biopsies from these patients were 'snap frozen' in liquid nitrogen and then sectioned at right angles to the horizontal axis of the skin on a Bright's cryostat at 6–15 μm. The following enzyme histochemical tests were performed on the biopsies of lichen simplex (Table 1).

In order to assess the relationship between the degree of epidermal thickening and the histochemical reaction observed, the following epidermal measurements were made:

The ratio of the length of the basal layer to the length of the granular cell layer (B/G ratio) was

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* Based on paper presented to the British Association of Dermatology, July 1972.

TABLE 1. A summary of the enzyme reactions performed and the controls used

Enzyme reaction	No. of biopsies	Method	Controls and inhibitors
Lactic dehydrogenase (LDH)	9	NBT method described in Pearse (1960)	Lactate excluded from medium
NADH diaphorase (NADH)	15	NBT method described in Pearse (1960)	NADH excluded from medium
Succinic dehydrogenase	12	NBT method described in Pearse (1960)	0·05 M sodium malonate inhibitor in medium
Glucose-6-phosphate dehydrogenase	8	NBT method described in Pearse (1960)	NADP excluded from medium
Cytochrome oxidase	3	Metal chelation method of Burstone (1959)	10^{-3} M KCN in medium
Beta-hydroxy butyric dehydrogenase	5	NBT method described in Pearse (1960)	NAD excluded from medium
Non-specific esterase	16	Indoxyl acetate method of Holt (1954)	Eserine sulphate ($3 \cdot 1 \times 10^{-5}$ M) in medium
Acid phosphatase	1	Lead method of Gomori (1941)	0·01 M sodium fluoride in medium
DOPA oxidase	8	Method of Becker, Praver & Thatcher (1935)	10^{-3} M KCN in medium
ATPase	6	Method of Wachstein, Meisel & Niedzwiedz (1960)	*p*-chloromercuric benzoate in medium
Alkaline phosphatase	5	Azodye method in Bancroft (1967)	Naphthol AS-TR phosphate excluded from medium

calculated from direct measurements of standard photomicrographs. Any areas that had been inadvertently cut obliquely were excluded from these measurements as the *B/G* ratio obtained from these sites would have been inaccurate.

The thickness of the epidermis was calculated directly from the standard photomicrographs, by counting the number of cells between the basal zone and the top of the granular cell layer at twenty random sites.

RESULTS

Mitochondrial enzymes

In general the distribution of the reaction products followed that seen in the normal epidermis, with more marked reactivity in the basal layer for the succinic dehydrogenase and NADH reactions. There was a slight increase in the density of the reaction products from these enzyme reactions in the thickened epidermis of the biopsies examined. This was especially marked with the succinic dehydrogenase reaction. In Table 2 the densities of the reaction products in areas of lichen simplex have been compared with those observed in normal epidermis. The glucose-6-phosphate dehydrogenase reaction was stronger in the region of the granular layer.

An attempt was made to correlate the degree of enzyme reactivity with the *B/G* ratio and the epidermal thickness. In Fig. 1 these correlations have been plotted for the NADH and LDH reactions, and it can be seen that no clear relationship emerges. In many of the biopsies the papillary capillaries were quite strongly reactive in these tests, especially with the LDH and NADH reactions.

Lysosomal enzymes

The non-specific esterase was marked in the region of the granular cell layer and appeared to be

TABLE 2. Mitochondrial enzymes

Enzyme reaction tested	Density of reaction products in lichen simplex compared with normal epidermis			
	Increased	Equal	Decreased	Totals
NADH	6	9	0	15
LDH	4	4	1	9
Succinic dehydrogenase	10	1	1	12
Cytochrome oxidase	0	3	0	3

stronger than normal; 'the esterase band' was also broader than usually seen in healthy skin. For this study we used the indoxyl acetate method because we have found previously that other methods for non-specific esterase activity give less consistent results within the epidermis. The acid phosphatase reaction showed essentially the same findings as for the non-specific esterase reactions.

DOPA oxidase

A normal number of DOPA positive cells was identified in the eight biopsies in which this reaction was performed. They were evenly distributed and in the form of large dendritic cells.

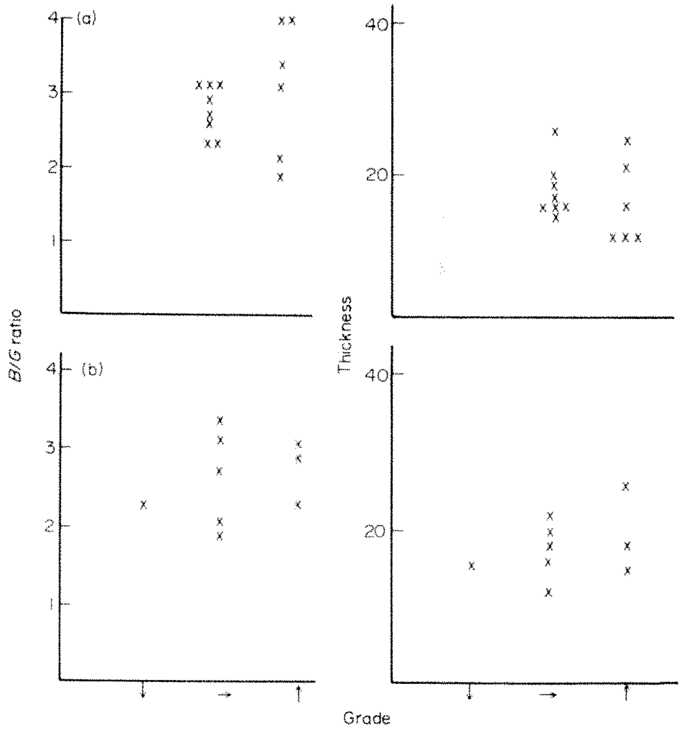

FIGURE 1. Diagrammatic representation of relationships between the basal layer/granular layer (*B/G* ratio), the epidermal thickness cells, and the density of reaction products in (a) the NADH diaphorase and (b) the LDH reactions (↓, decreased reaction product; →, normal reaction product; ↑, increased reaction product).

Alkaline phosphatase

The endothelial lining of the papillary capillaries was well seen with this reaction and demonstrated that these vessels were elongated and hairpin-like. They were less tortuous than the papillary capillaries of psoriatic lesions (McKenzie, 1963).

ATPase

The vascular endothelium also was easily seen when this reaction was performed. In addition many intraepidermal dendritic cells were seen (Fig. 2a) and these dendritic cells appeared increased in number. Small cutaneous nerves were identified in some specimens in the papillary corium (Fig. 2b). Apart from lengthening along with the papillary elongation their number appeared normal.

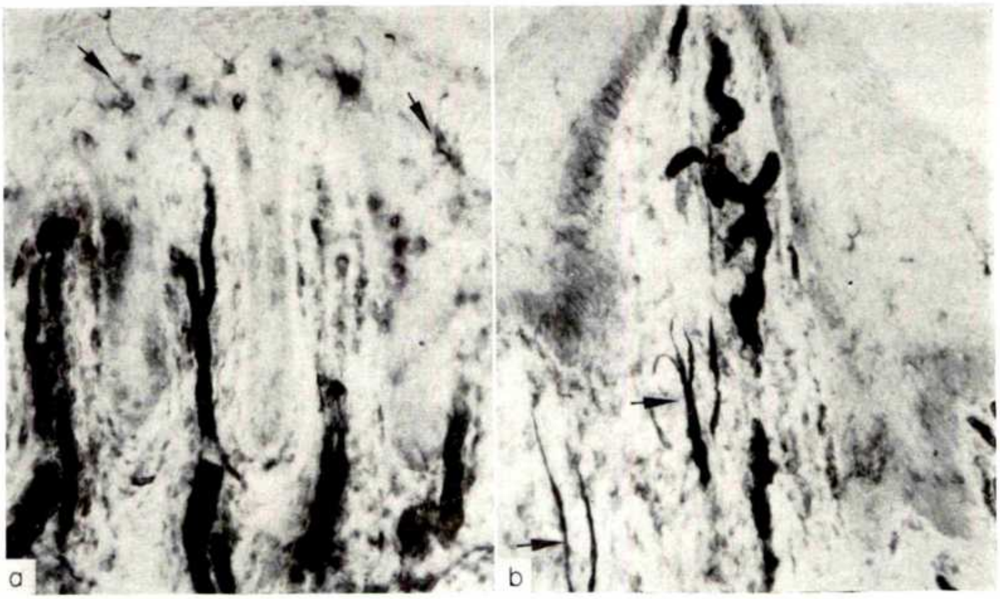

FIGURE 2. (a) ATPase reaction in lichen simplex. Note that there are many ATPase positive dendritic cells within the epidermis (arrowed). In addition, the papillary capillaries are well demonstrated (× 72). (b) ATPase reaction in lichen simplex. In this specimen many small cutaneous nerves are visible (arrowed) (× 72).

DISCUSSION

Lichenification has clinical and histological similarities with lichen planus and psoriasis. Despite the similarities the studies described in this paper indicate that there are also important histochemical differences. In particular lichen planus and lichen simplex have many distinguishing histochemical features. The investigations of Black & Wilson-Jones (1972) showed that the acanthotic epidermis of lichen planus had a markedly decreased reactivity for many of the mitochondrial enzymes, including NAD diaphorase, lactic dehydrogenase and succinic dehydrogenase. As we have demonstrated here, the hypertrophied epidermis of lichen simplex shows an increase in the mitochondrial enzyme reactivity, especially with the succinic dehydrogenase reaction. Black & Wilson-Jones (1972) also demonstrated that in lichen planus the pentose shunt enzymes showed a broadened area of reactivity corresponding with the hypergranulosis; this was not the case in the present studies of lichen simplex.

The presence of a normal number of DOPA positive cells in the basal part of the epidermis in lichen simplex also contrasts sharply with the findings of the previous authors of a complete absence of these cells in lichen planus. Necrosis of cells in the basal layer of the epidermis seems to be a specific feature of lichen planus and the observations suggest that melanocytes as well as keratinocytes are liquidated. Such cell necrosis is not seen in lichen simplex. Trauma may indeed produce cell damage within the epidermis in this condition, but not the peculiar basal damage of lichen planus.

There have been several reports of histochemical investigations of psoriasis, many of which have dealt with one aspect or another of the energy exchange pathways. Braun-Falco & Petzoldt (1967) were particularly impressed with an apparent increase in reactions depending on glycolytic enzymic pathways that they observed in psoriatic lesions. On the other hand, Gordon & Johnson (1967) found that the most significantly increased respiratory enzyme activity within the affected area was that of succinic dehydrogenase, an enzyme of the oxidative citric acid cycle. There are in addition many biochemical studies that all tend to confirm an overall increase in enzymes concerned with energy exchange within the psoriatic epidermis (Halprin & Taylor, 1971). The increased reactivity for this group of enzymes that we have observed in lichen simplex is of the same order as that in psoriasis and may be a reflection of the accelerated maturation of keratinocytes seen in both conditions. In contrast to psoriasis, lichen simplex shows a constant broad band for non-specific esterase activity in the superficial part of the epidermis. It follows the granular layer but is not confined to granulocytes, and in lichen simplex may be seen proximal to the granular layer. This 'band' was consistently present in our material and its increased width was not related to sectioning at an oblique angle. At present we can offer no explanation as to its significance. The non-specific esterase 'band' is broader and better defined in lichen simplex than in lichen planus and it is, of course, not present as a discrete 'band' in psoriasis.

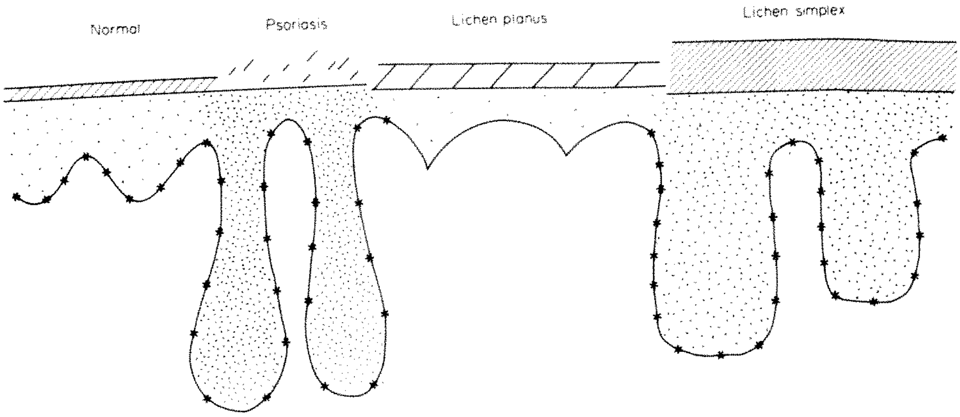

FIGURE 3. Diagramatic representation of some of the histochemical differences between normal skin, psoriasis, lichen planus and lichen simplex. Stippled shading, mitochondrial enzyme reactions; *, DOPA positive cells; diagonal shading, non-specific esterase reaction.

In Fig. 3 we have summarized and contrasted some of the histochemical findings in normal skin, lichen planus, psoriasis and lichen simplex. Some of the differences in the histochemical profiles may assist in understanding the structural differences observed in these dermatoses.

Jarrett & Spearman (1964) performed extensive histochemical tests on normal skin, psoriatic lesions, animal skin and induced parakeratosis in order to learn about the parakeratotic process. One finding of these authors is of particular interest to us as it concerns intra-epidermal ATPase positive cells in psoriasis. They found that ATPase positive cells were not present in increased numbers and seemed to be deficient in dendritic processes. In lichen simplex the intra-epidermal ATPase positive cells were prominent and appeared increased in number. Histochemical findings in lichen simplex appear to point to the hypertrophy of several anatomical components of the epidermis, while in psoriasis hyperplasia appears to be almost confined to keratinocytes.

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Application of the freeze-fracture replication technique to investigative dermatology

A.S.BREATHNACH

Department of Anatomy, St Mary's Hospital Medical School, London, W.2

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SUMMARY

The principles and practice of the freeze-fracture replication technique are outlined, and present progress relating to skin is summarized. The probable future value of the technique for the investigative dermatologist is assessed.

It seems clear now that the technique of freeze-fracture replication is capable of providing greatly extended insights at the ultrastructural level into certain aspects of the functional morphology of biological tissues (Steere, 1957; Koehler, 1968; Branton, 1971; Moor, 1971). Some enthusiasts would even go so far as to predict that its ultimate effect on fundamental concepts of cellular structure will be as great as that resulting from the introduction of conventional transmission electron microscopy of thin sections. The emergence of such a new technique, with apparently revolutionary potentialities, presents a number of problems to different people. The already established and well tooled-up basic or applied investigator must decide if, indeed, it is really necessary for him to practice the new art, and to become involved once again in the laborious process of selling himself and the method to the powers-that-be who control the necessary finance. Editors and referees will likely have to deal with a surge of manuscripts difficult to comprehend and assess, and finally, the non-specialist reader of the journals will have to decide if the attempt to keep up to date with the new development is really worth the effort; even if he could understand what it is all about. Skin is, as yet, practically unexplored by this technique, and as far as can be determined at the time of writing, the entire published literature in the field consists of two papers and an abstract (Reed, Kennelly & Boyes, 1968; Reed & Rothwell, 1970; Breathnach, Goodman, Stolinski & Gross, 1972a). Since this situation seems unlikely to endure for very much longer, it may be helpful to present a brief account of the principles and practice of the technique, with a view to attempting an assessment of its probable value in the field of investigative dermatology, both basic and applied.

PRINCIPLES AND PRACTICE OF THE TECHNIQUE

General principles

A major advantage of the technique is that it provides a method for observing the ultrastructure of essentially viable tissue which has not been exposed to chemical fixation, dehydration, infiltration with embedding media, sectioning, or staining, all of which procedures may give rise to artifact. Fresh tissue is rapidly frozen to −160°C, or lower, fractured *in vacuo*, and a shadowed replica of the exposed surface is then obtained by evaporation of a heavy metal (usually platinum), and carbon. The replica is then separated from the tissue, placed upon a grid, and examined in the electron microscope. It will be clear that the fracture reveals two surfaces, one on each piece of tissue which it separates, and, depending upon the apparatus, one or both surfaces may be replicated. Since, in theory at any rate, they should match perfectly, for many purposes it is only necessary to replicate one surface. Resolution of the order of 2 nm may be achieved with good replicas, and it is probable that this will soon be improved.

The fracture traverses the cytoplasmic matrix of a frozen cell in an apparently random fashion, and the two resulting fracture faces appear identical. As it approaches membranes, however, it frequently deviates, and runs parallel with the membrane for variable distances, thus exposing extensive areas *en face*. Membranes may also be fractured straight across, thus appearing as they would in thin sections (Figs. 1 and 2). It is this more extensive revelation of membranes (plasma and nuclear membrane, and membranes of organelles) which renders the technique so potentially valuable. Deviation of the fracture as it traverses the tissue, combined with the effect of shadowing, leads to a three-dimensional appearance in micrographs.

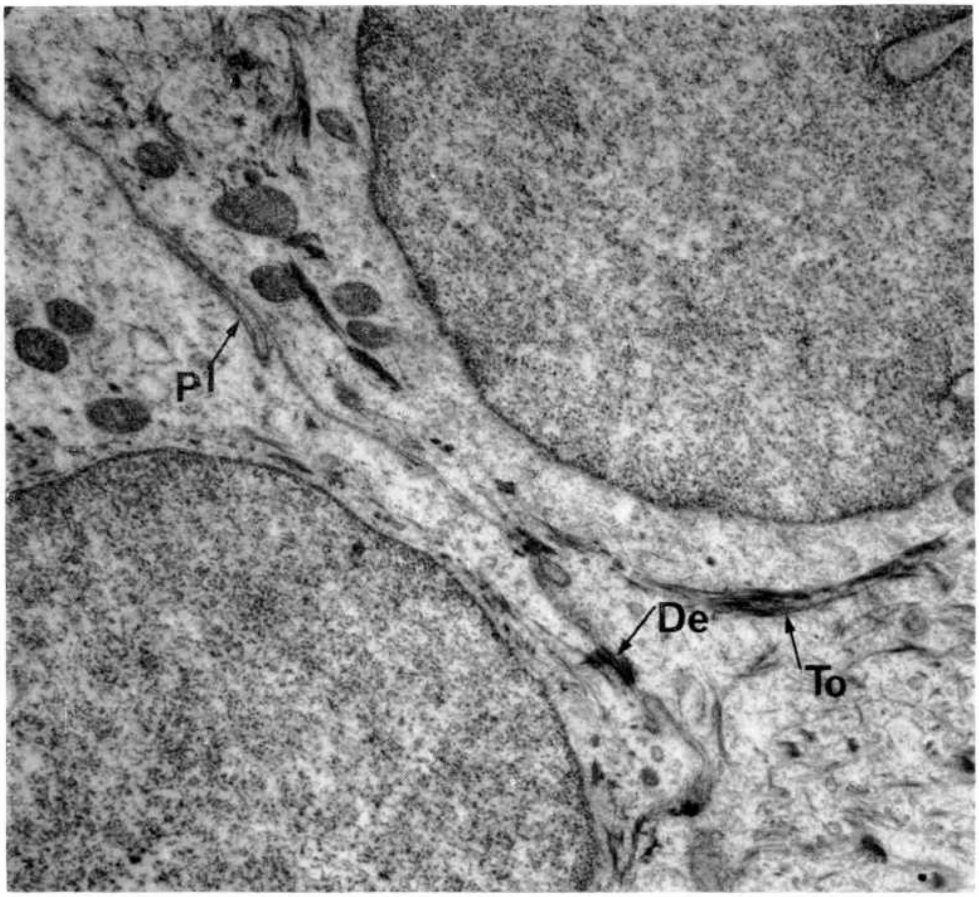


FIGURE 1. Stained thin section of chemically-fixed human epidermis for comparison with replica shown in Fig. 2. Nu, nucleus; Pl, apposed plasma membranes of two cells; De, desmosome; To, tonofilaments (× 20,000).

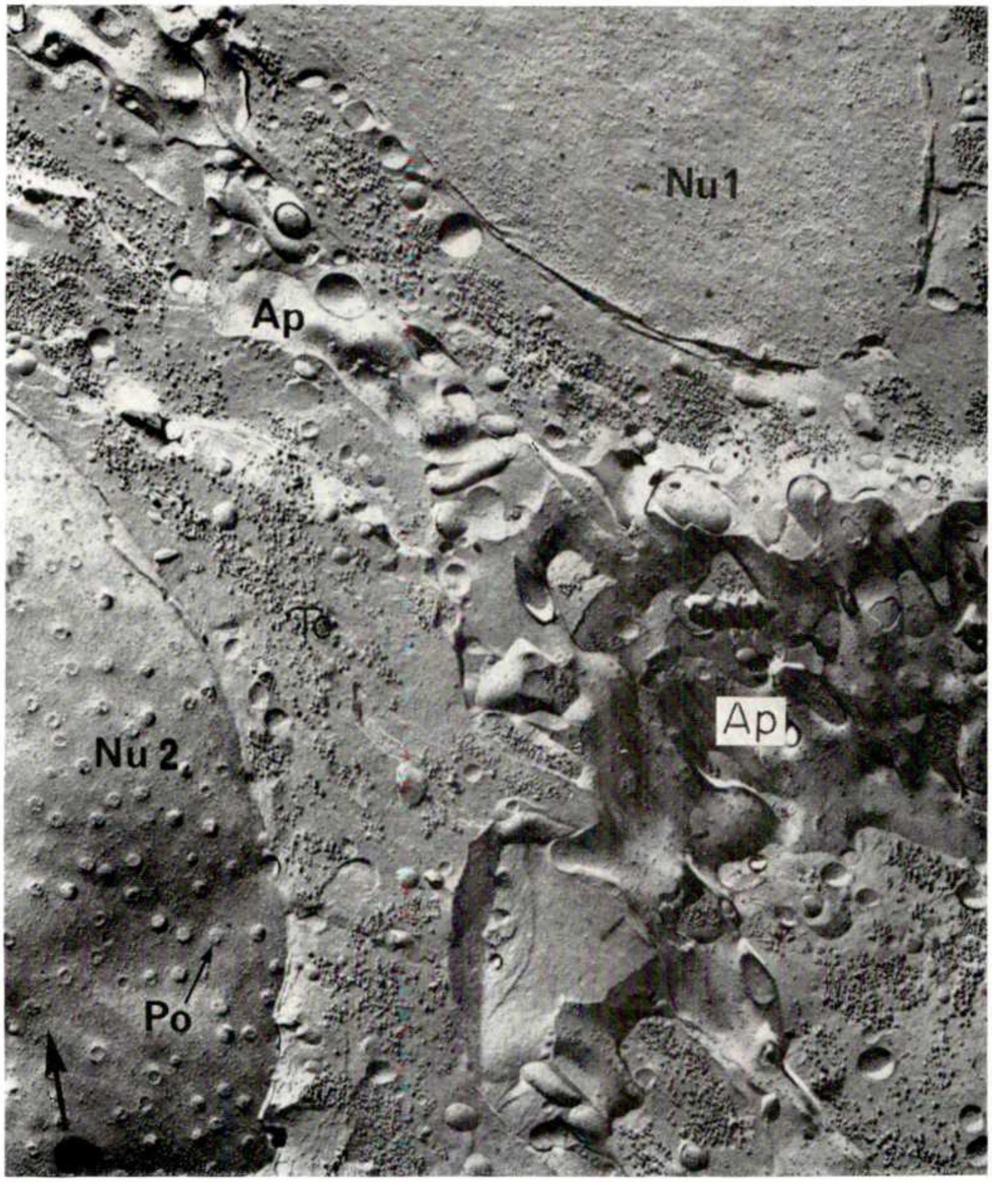


FIGURE 2. Freeze-fracture replica of epidermal keratinocytes for comparison with Fig. 1. The nucleus of one of the cells shown (Nu 1) has been cross-fractured, and appears essentially similar to nuclei seen in the thin section. The other nucleus (Nu 2) has been fractured so as to reveal an *en face* view of the inner nuclear membrane, and nuclear pores (po) are a prominent feature of this fracture face. Ap, regions of cell apposition revealing fracture faces of apposed membranes, and presenting a most complicated appearance due to interlocking of villous processes; To, tonofilaments (× 20,000) (Breathnach, 1973a).

Note: In this, and succeeding micrographs, the direction of platinum shadowing is indicated by the large arrow.

What has been described so far, is plain 'freeze-fracturing'. In certain circumstances the overall surface of the tissue exposed by the fracture may be further 'etched' before replication. This is the process of 'freeze-etching', and it involves sublimation of a controlled depth of ice away from the general surface, thereby lowering it, leaving non-aqueous components protruding more prominently, and revealing surfaces previously obscured. 'Etching', or more correctly 'sublimation', has certainly proven to be a most valuable procedure, when applied to isolated cells suspended in aqueous media (Branton, 1971) but its application to organized tissues presents some difficulty, as will be explained later.

Apparatus

The procedures outlined above require complicated and highly sophisticated apparatus, details of which cannot be given here. Essentially it consists of an efficient high vacuum pumping system combined with a unit in which the tissue may be fractured and replicated under controlled conditions of pressure and temperature. Given a basic pumping unit, which most electron microscope laboratories already possess, and adequate design and workshop facilities, it is possible to equip oneself at relatively low cost, and the replicas used to illustrate this article were produced in a unit designed and built by Mr C. Stolinski of the Department of Biophysics of this medical school. A number of efficient purpose-designed machines are already available commercially, and, no doubt, these will be the choice of most prospective operators who can afford them.

Possible sources of artifact

Whereas, as already mentioned, certain sources of artifact to which conventionally processed tissue is prey are eliminated with the freeze-fracture technique, there are others which must be considered. Most important of these is crystallization of ice within the tissue, which can lead to damage or distortion. This can largely be controlled by rapid freezing and precise temperature control, and the use of cryoprotective agents. Glycerol (20–25%) is the most commonly used cryoprotective, but its use prevents effective 'etching', since adequate sublimation of glycerinated tissue cannot be achieved. Other cryoprotectives, such as dimethyl sulphoxide which will permit sublimation, are being investigated, and preliminary results are encouraging. Contamination of the fracture surface by oil or water vapour, or by fragments of tissue fractured away can occur, but again these sources can be controlled, and, it is hoped, finally eliminated, by improvements in design of the apparatus. Finally, the replica itself may be contaminated by remains of tissue adhering to it. This is a particular danger with skin, which is very difficult to separate from the replica. It has to be completely digested away with strong acids and alkalis, and collagen and keratin present major problems in this respect.

It is necessary to stress this question of artifact since it is probable that other sources and effects remain to be identified and documented, and that some detailed features of replicas may not have the significance attributed to them at the moment. Nevertheless, the reality of many of the features revealed is now well established, and it is possible to make valid and useful comparisons between replicas and thin sections which will permit evaluation of the particular advantages of the new technique for studying individual tissues. Establishment of normal appearances is an essential basic requirement, and good progress in this direction has lately been achieved for epidermis and for some dermal elements (Breathnach, Stolinski & Gross, 1972a; Breathnach, 1972; Breathnach, Goodman, Stolinski & Gross, 1972b). With one exception (Fig. 8), the micrographs which illustrate this paper show replicas of tissue which was freeze-fractured, but not 'etched'.

EPIDERMIS

General features

Figures 1 and 2 present respectively a stained thin section and a freeze-fracture replica of epidermal

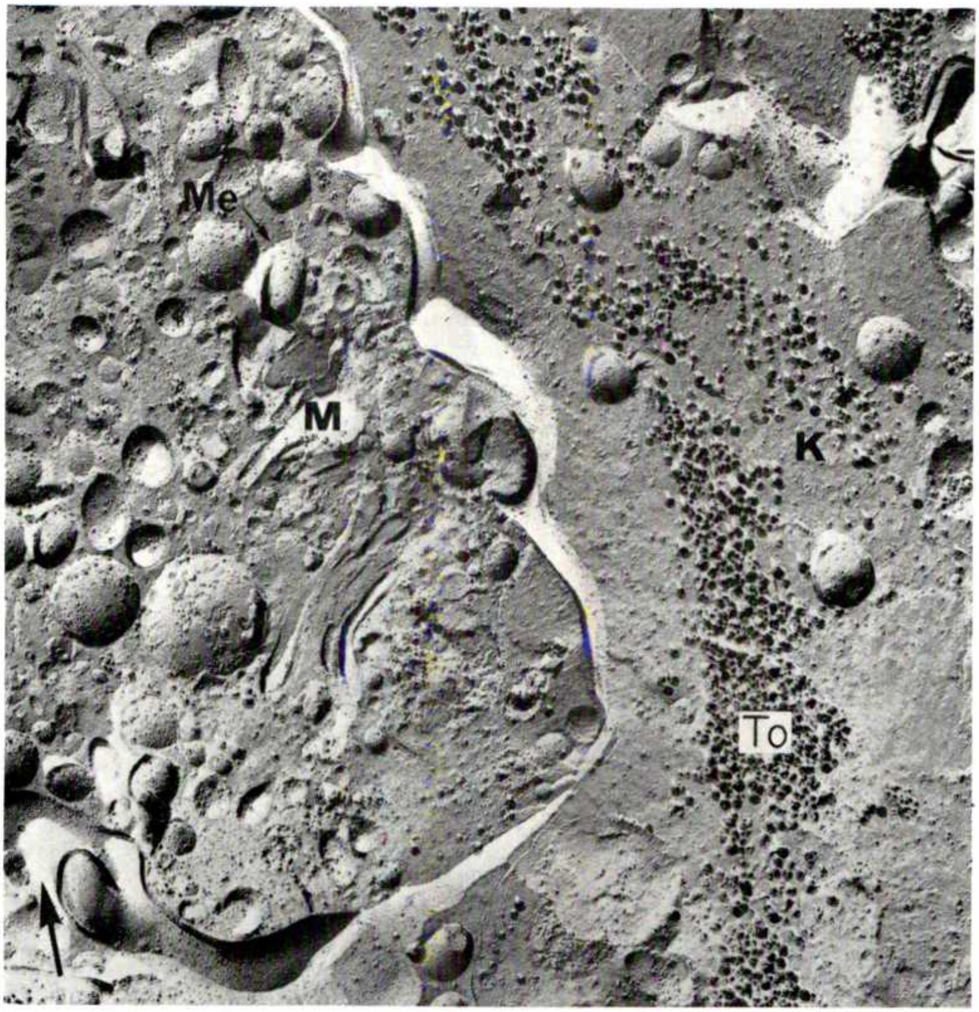


FIGURE 3. Freeze-fracture replica from basal layer of human epidermis. On the right is the cytoplasm of a basal keratinocyte (K) with characteristic tonofilaments (To). On the left is portion of a basal melanocyte (M). Note absence of tonofilaments from this latter cell, and presence of melanosomes (Me) (×35,000) (Breathnach, 1973b).

keratinocytes for comparison. Of the two nuclei seen in the replica, one has been cross-fractured so as to reveal the nucleoplasm, and an 'edge-on' view of the nuclear membrane. Its appearance, in fact, is essentially similar to the appearance of the nuclei in the thin section. The other nucleus, however, appears very different. It has been fractured so as to reveal an *en face* view of one of the nuclear membranes, the inner one. Nuclear pores are a prominent feature of this fracture face, and it is evident that this type of preparation can permit study of the pattern of distribution and numerical density of these pores, an exercise which is not feasible with thin sections, even if a goniometer stage is available. It seems reasonable to suggest that these features might vary during the life-span of a cell, and the epidermis provides an ideal situation for investigating this proposition. It contains in orderly sequence, from the basal to the granular layer, cells of progressively increasing age. Patterns and

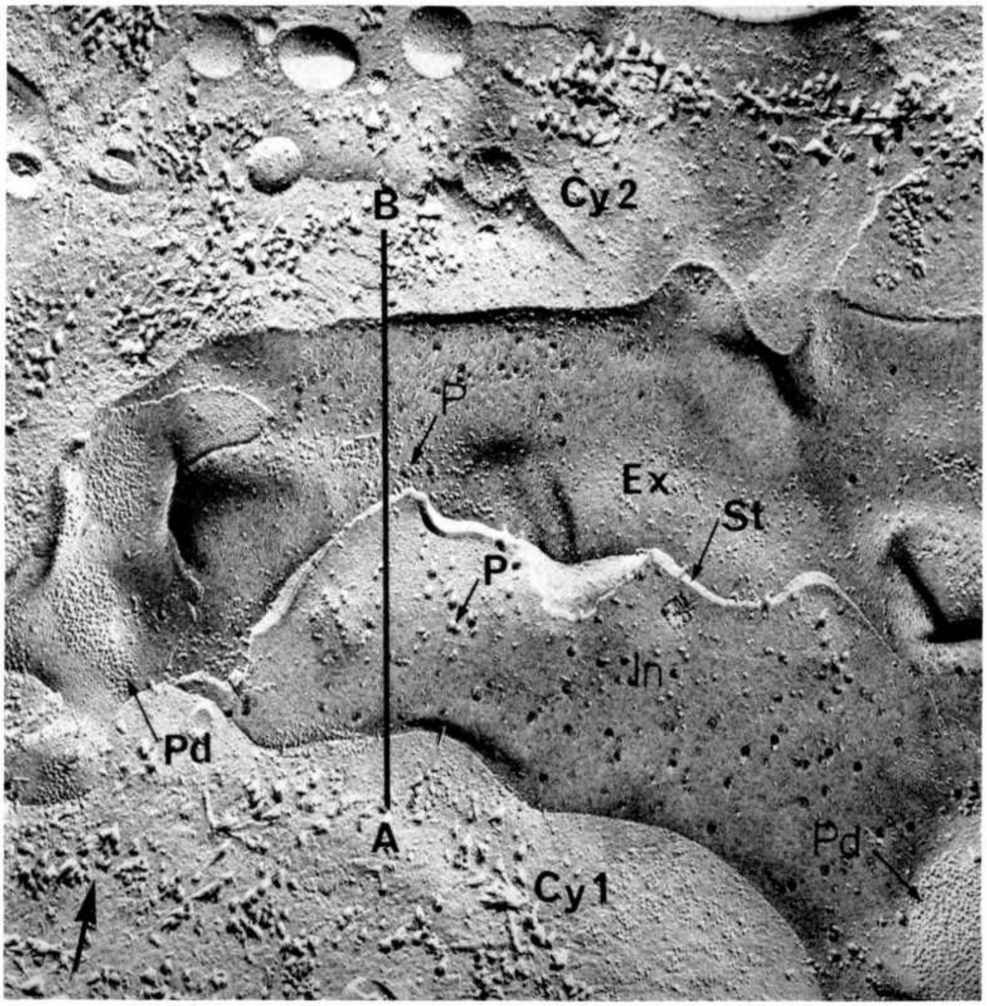


FIGURE 4. Freeze-fracture replica of apposition of two cells in basal layer of human foetal epidermis. Proceeding along the line from A to B, the fracture reveals in succession: cytoplasm of one cell (Cy 1); a fracture face of the plasma membrane (In) directed towards the interior of the cell; a step down (St) which includes the intercellular material; a fracture face of the plasma membrane of the apposed cell (Ex) which is directed towards its exterior; the cytoplasm (Cy 2) of this second cell. Note isolated particles (P) of varying size on both fracture faces In and Ex; these are 'membrane associated particles'. Note also on fracture face In exclusively, aggregations of closely-packed particles (Pd) which coincide with desmosomes (× 58,500).

numbers of nuclear pores might also vary under physiological, experimental, or specific disease conditions, and likewise could be investigated in these particular instances.

Tonofilaments are equally evident within the cytoplasm of cells processed by the different techniques. In thin sections they may appear cut transversely, or as bundles of varying length lying parallel to the plane of section. In replicas, they appear most commonly to be cross-fractured, standing out prominently above the general fracture plane (Figs. 3 and 4) which suggests they may be pulled slightly out of the cytoplasm before they fracture (Breathnach *et al.*, 1972a). Filaments disposed parallel with the plane of fracture would doubtless be revealed by sublimation. Estimated diameter of the tonofilaments in replicas, both at lower levels and in the stratum corneum (Breathnach, Goodman *et al.*, 1972b) is 10 nm, a somewhat higher figure than that derived from measurements on thin sections. The absence of tonofilaments from melanocytes is also clearly shown in replicas (Fig. 3), but other features of this class of cell have not been investigated to date. The melanocyte has been so thoroughly worked over by generations of electron microscopists, that it is almost inconceivable that any new technique could provide further information concerning its ultrastructure. However, in so far as freeze-fracture can reveal different internal fracture faces of organelles, uncomplicated by heavy-metal staining, it may extend understanding of the structural organization of the normal melanosome, and of the morphological variants produced by the malignant melanocyte. The characteristic organelle of the Langerhans cell could also bear investigation.

Vesicular elements of concave or convex appearance present in the cytoplasm represent mainly mitochondria. Rough endoplasmic reticulum is not prominent in epidermal cells, but it has a highly characteristic appearance in replicas (Breathnach *et al.*, 1972b).

Plasma membrane

In thin sections, apposed plasma membranes of adjacent cells are seen sectioned edge-on, with desmosomes at intervals along their lengths (Fig. 1). At various times, and in particular with pathological tissue, authors have attempted to pronounce upon the shape, size, and numbers of desmosomes from study of this type of preparation. This is really not feasible, even with serial sectioning, which is rarely employed. In replicas, by contrast, extensive areas of apposed membranes are seen *en face*, and in order to appreciate the significance of their features, it is necessary to be quite clear as to what these areas represent. This should become evident from examination of Fig. 4, which features an apposition of two keratinocytes. If one follows the path of the fracture along the line AB, it can be seen to have revealed in succession, (a) the cytoplasm of one cell, (b) a fracture face of the plasma membrane of this cell (In), which is directed towards its interior, (c) a step (s), which includes the intercellular material, (d) a fracture face of the plasma membrane of the apposed cell (Ex), which is directed towards its exterior, and (e) the cytoplasm of this second cell. Originally, it was thought that the faces In and Ex represented, respectively, the true inner and outer surfaces of the plasma membranes concerned. However, it is now generally agreed that the fracture traverses the *interior* of the membrane, and that the faces revealed are internal or 'split' faces of the membrane. This means that the isolated particles—'membrane-associated particles' (p)—revealed on the fracture faces, represent internal structural components of the membrane (Branton, 1971; Moor, 1971). Such particles are seen on fracture faces of plasma membranes of all tissues, and much fundamental work is being carried out to determine their exact nature and significance. The results of this work will obviously have important applications in connection with concepts of basic membrane structure, which must be taken into account by investigators of all tissues. In addition to the isolated particles, localized aggregates of closely packed particles (Pd) are seen on fracture face (In) of the plasma membrane. These occur at desmosomal sites and are co-extensive with them (Breathnach *et al.*, 1972a; Breathnach, Goodman *et al.*, 1972b). When extensive areas of this type of fracture face are revealed (Fig. 5), it is evident that a realistic evaluation of the shape, size, and density of distribution of desmosomes can be achieved, even though the precise significance of the aggregated particles is not yet apparent. All one can say at the moment is, that they are located within the plasma membrane at desmosomes, and that on fracturing, they remain attached to the fracture face of the membrane (In), which is directed towards the cytoplasm. In stratum corneum, aggregated desmosomal particles are present on both fracture face In and face Ex of the plasma membrane, but membrane-associated particles are virtually absent from the rest of the membrane (Breathnach, Goodman *et al.*, 1972a, b). These differences are

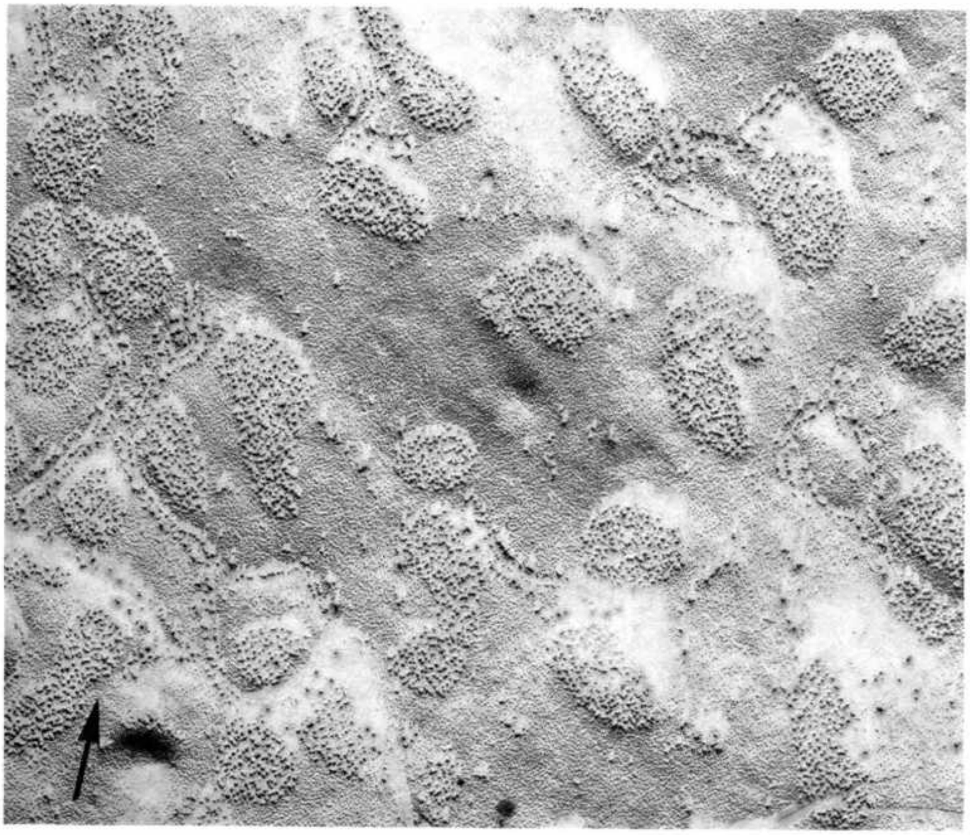


FIGURE 5. Freeze-fracture replica showing fracture face In of periderm cell of human foetal epidermis. Aggregated desmosomal particles are numerous on this fracture face, and it is evident that this type of preparation allows one to estimate the density of distribution of desmosomes per unit area of plasma membrane. Variations in size and shape of desmosomes are also clearly shown (× 62,000).

presumably indicative of alterations in structure of stratum corneum plasma membrane, and whereas, again, their exact significance is not apparent at present, their demonstration makes it well worth while examining plasma membranes at all epidermal levels with this technique, in conditions such as psoriasis, where the normal process of keratinization is affected.

Gap junctions and tight junctions have been extensively studied in replicas of other tissues (Scott, McNutt & Weinstein, 1970; Goodenough & Revel, 1970; Friend & Gilula, 1972) and their appearance in epidermis is essentially similar. These studies have advanced understanding of the functional morphology of these specialized contacts.

Other features

Problems of the origin, nature, and significance of keratohyalin remain largely unresolved despite extensive studies over the years. In view of this, the appearance of keratohyalin in freeze-fracture replicas should prove of interest. Well, the position is, that to date, nothing which could positively be equated with a deposit of keratohyalin has been seen in any replica of stratum granulosum (Fig. 6). This could lead to a particular conclusion, though one hesitates even to suggest it. Another well-

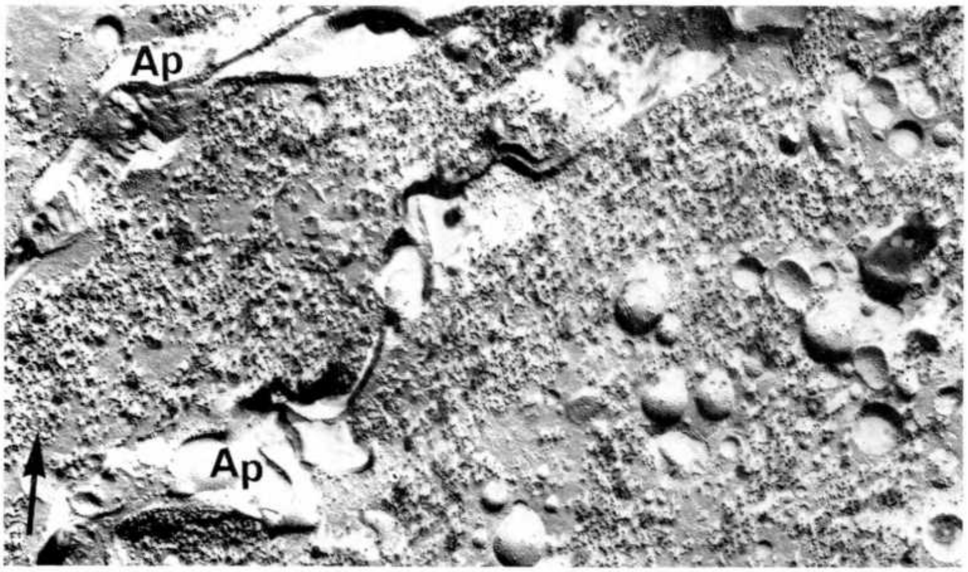


FIGURE 6. Freeze-fracture replica of stratum granulosum of human epidermis. Ap, apposition of cells. Nothing which can positively be identified as keratohyalin is present in the cytoplasm (×36,000).

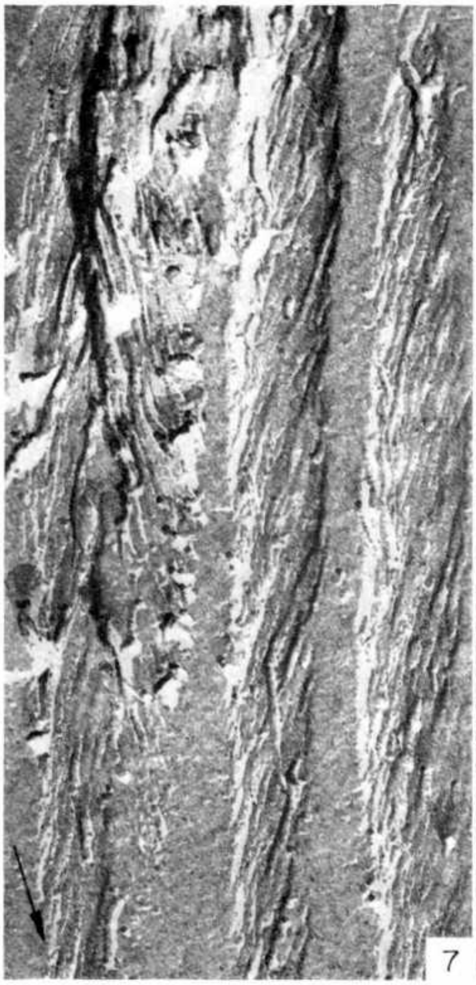
known feature of skin, the basal lamina at the epidermal–dermal junction, has likewise not yet been demonstrated as a distinct, limited, entity in replicas (Breathnach *et al.*, 1972a). To some extent this failure to visualize the two structures in question may be attributed to their presumed largely 'amorphous' nature, and it may be that sublimation, when it becomes practicable, will reveal some evidence of their presence. Lamellar granules (membrane-coating granules) are readily demonstrable in replicas (Breathnach, Goodman *et al.*, 1972b).

Epidermal appendages, i.e. hairs and glands, have not yet been submitted to examination by the freeze-fracture technique, but considering what it can reveal, as indicated above, this should prove a highly rewarding field for future investigation.

DERMIS

Of the basic dermal elements, such as collagen, elastin, nerves and vessels, it is only the latter two that have been examined to any extent, and then only in tissues other than skin. It is too early, as yet, to pronounce upon the probable value of investigating terminal vessels and nerves of skin by this technique, though on the general ground that these elements may be affected in a variety of skin diseases, it would seem desirable. In unsublimated material, collagen fibres (Fig. 7) present a characteristic 'twisted-rope' or helical appearance similar to that demonstrated by Bouteille & Pease (1971) in material prepared by 'inert dehydration'. These authors believed this appearence to be due to helical organization of unit filaments, and rejected a suggestion (Szirmai, van Raamsdonk & Galavarzi, 1970) that the torsion might be derived from a sheet of material coating the fibres.

However, that this may be the case could be suggested by the fact that in sublimated material the helical arrangement is rarely seen; instead, the banding characteristically seen in thin sections is revealed (Fig. 8). This difference in appearance, whatever its true significance, does serve to under-



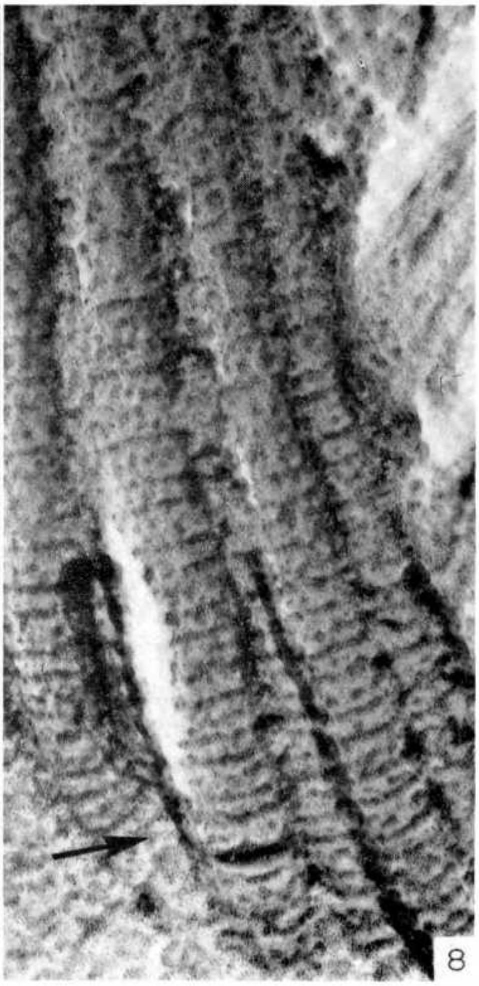


FIGURE 7. Freeze-fracture replica of collagen fibres from human dermis. Note characteristic 'twisted-rope' or helical appearance of the fibres in this unsublimated material (× 150,000).

FIGURE 8. Replica of collagen fibres from human dermis. Sublimation of the fracture surface was performed before replication. Note, in comparison with Fig. 7, the evident banding of the fibres (× 140,000).

line the fact that a distinction should always be made between non-sublimated and sublimated material, something which many authors fail to do. Also, since sublimation involves an additional step beyond the primary freeze-fracture, and since its effects are not yet fully documented, it is advisable always when dealing with a tissue not previously studied, to establish the uncomplicated freeze-fracture appearances, before embarking upon sublimation.

Micrographs of replicas of elastic fibres of skin have only very recently become available (Fig. 9) and apart from observing that the fibrillar component is well visualized, it is not possible to make further comment at the moment. However, even on the slight evidence available to date, application of the technique to the study of connective tissues seems likely to supplement the information available from study of conventional thin sections.

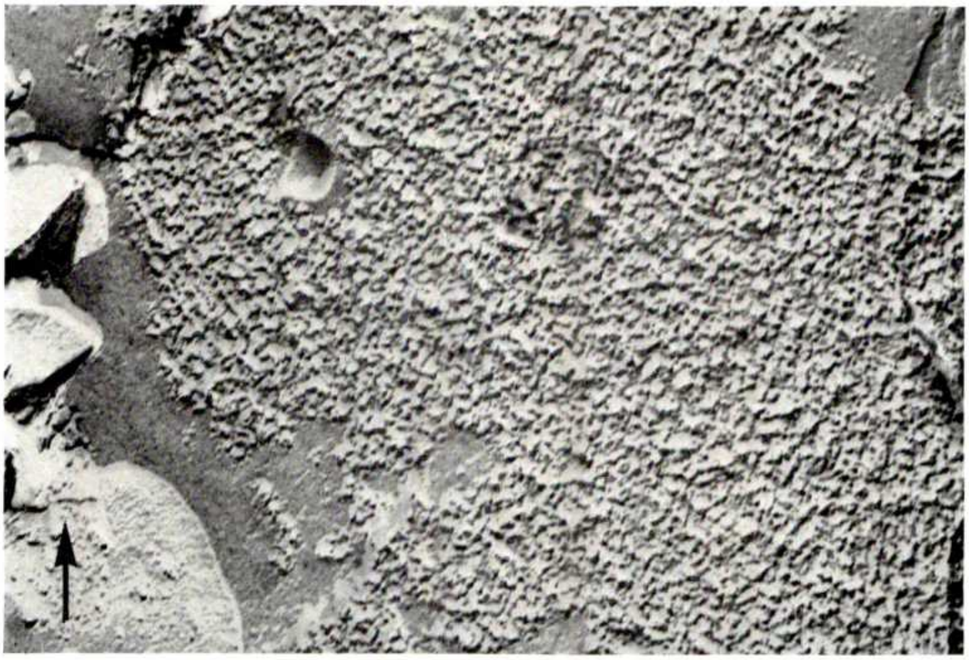


FIGURE 9. Freeze-fracture replica of elastic fibre from human dermis. The fibrillar substructure is clearly evident (×84,000).

CONCLUSIONS

Considering the present state of the art, and its minimal application, so far, to skin, it might be considered premature to attempt an assessment of the value of the freeze-fracture technique to the investigative dermatologist. It could be said that, until the basic workers operating at the macromolecular level are confident they can separate artifact from reality, and can equate what they can demonstrate with known chemical and biophysical data, others concerned with higher levels of structural organization, should mark time. Certainly, the greatest impact of the technique will be upon current concepts of basic membrane structure, and anyone concerned with whatever aspect of the functional morphology of membranes at the molecular level, must take account of it. However, it is suggested that the few examples cited here justify its immediate application at the somewhat higher level of what may be termed general ultrastructural topography. There is no doubt that at this level the technique can be a valuable supplement to conventional techniques of transmission electron microscopy, in that it can provide another dimension for the examination of well-established and recognizable features, and in a condition very close to the living state.

ACKNOWLEDGMENTS

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Hydrolysis of β-naphthyl acetate by plasma and by skin extracts from normal and atopic subjects

D.W.K.COTTON, JOSÉ J.M.A.VAN DEN HURK AND P.D.MIER

Department of Dermatology, University of Nijmegen, The Netherlands

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SUMMARY

The hydrolysis of β-naphthyl acetate by plasma and by skin extracts has been investigated. In both cases the specimens from normal and atopic subjects yielded similar kinetic data.

These results do not support the hypothesis of an abnormality in the enzyme acetylcholinesterase in the atopic.

The atopic state is often described as one in which the subject displays an inbalance in the autonomic nervous system (Russell & Last, 1955; Mali, 1971). In general it appears that the sympathetic tone is reduced and that the parasympathetic tone is increased. This impression has been embodied in an elegant theory (Szentivanyi, 1968) in which it is suggested that the β-adrenergic receptors are in some way deficient. The β-receptors have been identified as the membrane-bound enzyme adenyl cyclase; this catalyses the production of Sutherland's 'second messenger', cyclic AMP, which in turn activates a cascade of intracellular enzymes (Sutherland & Robinson, 1966). Despite the convincing nature of Szentivanyi's arguments, his original model does not seem to fit the situation in atopic dermatitis, since kinetic studies of these enzymes have revealed no difference between normal and atopic skin (Mier & Urselmann, 1970; Holla *et al.*, 1972; Mier & van den Hurk, 1972).

It has already been pointed out, however, that the data presented by Szentivanyi will fit a number of other models equally well (Cotton, 1970; Cotton, 1972). In view of our inability to demonstrate any impairment of the β-adrenergic response, we have consequently turned our attention to the alternative possibility; that is, the existence of a defect which leads to the overstimulation of the parasympathetic aspect of the autonomic balance. The most plausible defect of this nature would seem to be an impairment of the enzyme acetylcholinesterase, resulting in a delayed clearance of acetylcholine from the tissue. Such a concept is wholly compatible with reports that atopic subjects show anomalous responses to this compound; examples include excessive vasodilatation (Ramsey, 1969) and an exaggerated sweat response (Warndorff, 1970). Although an acetylcholinesterase deficiency was regarded as 'theoretically possible' by Russell & Last (1955), and has been touched upon by a number of subsequent authors, no quantitative kinetic study of this enzyme in atopic subjects has been reported.

In this and the following publication we wish to describe the results of two such investigations. The first of these utilized the synthetic substrate β-naphthyl acetate; this approach offers a simple, rapid and sensitive technique, the validity of which has already been established by model studies in the guinea-pig (Cotton & van den Hurk, 1972).

INVESTIGATION

Materials

Reagents were in general of analytical grade. β-naphthyl acetate was purchased from the Sigma Chemical Company Inc. This material gave a rather high blank, which tended to increase with storage; it was therefore recrystallized from aqueous ethanol prior to use.

Patients with clinically typical atopic dermatitis were either hospitalized or attending the out-patient clinic of this department. Patients with asthma were attending the Longcentrum Dr v. Spanje-Kliniek Groesbeek; control specimens were obtained from paid volunteers who had no personal or family history of an atopic disposition. In all cases skin biopsy was performed using a 4 mm punch after freezing the skin with ethyl-chloride spray. Blood was taken into heparin, and the plasma separated by centrifugation.

Assay

The technique employed utilized the synthetic substrate β-naphthyl acetate. This is split to β-naphthol and acetic acid; the β-naphthol is strongly fluorescent and its production can be continuously monitored.

A series of buffered substrates was prepared containing 0·05 mol/l tris (pH 7·2), 0·5 mmol/l EDTA, 30 mmol/l $MgCl_2$ and β-naphthyl acetate in the range 0·054–0·80 mmol/l. Skin biopsies were homogenised in 2 ml of a solution containing tris, EDTA and $MgCl_2$ in the concentrations described above, to which bovine serum albumin (10 mg/ml) had been added. The homogenate was centrifuged, and the residue discarded. The assay was initiated by the addition of 0·1 ml of skin extract to 0·4 ml of the appropriate buffered substrate in a quartz cuvette; in the case of plasma 0·02 ml of the plasma was added to 0·48 ml of buffered substrate. The cuvette was transferred to a fluorimeter ('Fluorispec', Baird Atomic) which was thermostatted at 37°C, and the fluorescence recorded until a linear plot had been established (excitation 315 nm, emission 420 nm). The sensitivity of the instrument and the chart speed of the recorder were adjusted to give a plot of about 45°.

Initial reaction velocities were calculated using a standard curve prepared from β-naphthol; day-to-day fluctuations in instrumental sensitivity were compensated by the use of quinine sulphate as a secondary standard. The reciprocals of the initial velocities were plotted against the reciprocals of the substrate concentrations (Lineweaver and Burk plot), and the intercept on the substrate axis used to calculate the apparent K_m (Michaelis–Menton constant) for the sample.

RESULTS

The rates of hydrolysis of β-naphthyl acetate under 'saturation' conditions (V_{max}) and of the substrate concentrations at which the reaction velocity falls to half-maximal (K_m) are shown for plasma in Table 1 and for skin extracts in Table 2.

In neither case do the kinetic constants show a significant correlation with disease state.

DISCUSSION

The results clearly provide no support for the concept of an abnormality of the enzyme acetylcholinesterase in the atopic individual.

Nevertheless, these negative findings must be qualified by the reservation that the technique employed is far from specific. Although providing an ideal method for the assay of purified acetylcholinesterase (Guilbault & Kramer, 1965), β-naphthyl acetate is also hydrolysed by a range of other carboxylic ester hydrolases found in plasma or crude tissue extracts. This lack of specificity does not,

TABLE 1. Hydrolysis of β-naphthyl acetate by plasma. The values of V_{max} are μmol/min/ml plasma, and the K_m is expressed as mmol/l

Specimen no.	Atopic		Non-atopic	
	V_{max}	K_m	V_{max}	K_m
1	0·06	1·9	0·15	1·9
2	0·68	7·2	0·07	2·1
3	0·17	5·0	0·33	8·3
4	0·07	2·3	0·13	4·6
5	0·46	1·7	0·50	1·3
6			0·33	8·3
7			0·10	3·7
Mean	0·29	3·6	0·23	4·3

TABLE 2. Hydrolysis of β-naphthyl acetate by skin extracts. The values of V_{max} are μmol/min/g fresh weight of skin, and the K_m is expressed as mmol/l

Specimen no.	Atopic		Non-atopic	
	V_{max}	K_m	V_{max}	K_m
1	18·8	0·62	16·0	0·55
2	9·4	0·50	14·0	0·50
3	24·0	0·50	7·9	0·66
4	24·0	0·42	15·2	0·50
5	29·0	0·56	19·2	0·51
6	18·0	0·48		
7	9·4	0·50		
Mean	18·2	0·51	16·5	0·52

per se, affect our conclusions, since we have made a direct comparison between atopic and non-atopic tissue. There remains, however, a marginal possibility that a small reduction in acetylcholinesterase activity in the atopic might be obscured by relatively high activities of other esterases. In addition, the nature of the assay did not permit an examination of particulate material; although no data are available for skin it is known that in certain tissues (e.g. erythrocytes) acetylcholinesterase exists largely as a membrane-bound enzyme.

In view of these limitations it was considered desirable to supplement the present conclusions by the use of an independent technique; this is reported in the following communication.

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Hydrolysis of acetylcholine by soluble and particulate preparations of skin from normal and atopic subjects

D.W.K.COTTON, JOSÉ J.M.A.VAN DEN HURK AND P.D.MIER

Department of Dermatology, University of Nijmegen, The Netherlands

Accepted for publication 28 November 1972

SUMMARY

The hydrolysis of acetylcholine by preparations of mouse and human skin has been studied. Activity has been demonstrated in both soluble and particulate fractions. In the former case at least two enzymes appear to be present; the K_m values suggest these to be 'true' (3.1.1.7) and 'pseudo' cholinesterase (3.1.1.8).

Biopsy specimens of skin from normal and atopic subjects yielded similar kinetic data. This supports our previous conclusion that a reduced rate of hydrolysis of acetylcholine is not associated with the atopic disposition.

In our previous communication (Cotton, van den Hurk & Mier, 1973) we described the measurement of carboxylic ester hydrolases (including cholinesterases) using the synthetic substrate β-naphthyl acetate. No differences were found between normal and atopic subjects; however, the limitations inherent in this approach were discussed.

We now wish to report an extension of this work. The use of the physiological substrate, acetylcholine, has overcome possible objections concerning the specificity of the assay technique; in addition, by employing a radiometric rather than an optical assay, it has proved possible to examine both the soluble and the particle-bound cholinesterase activity of skin.

INVESTIGATION

Materials

Chemical reagents and clinical material were, in general, as described previously (Cotton *et al.*, 1973). All biopsy specimens for this work were taken from clinically healthy skin of the upper arm. Mice were adult albino animals; after killing by a blow to the head, a specimen of skin was shaved, removed, and dissected free of subcutaneous muscle.

(Acetyl-1-^{14}C) choline chloride was obtained from the Radiochemical Centre (Amersham, England), at a specific activity of 13·5 mCi/mmol. Free acetate was removed prior to use by dissolving in 0·1 N HCl, extracting three times with toluene/isoamyl alcohol (5:1), and neutralizing with NaOH.

Assay technique

The method of Potter (1967) was employed. This is based on a partitioning between dilute mineral acid and toluene; free acetate is extracted quantitatively into the organic solvent, whereas unchanged acetylcholine remains almost exclusively in the aqueous phase.

Buffered substrate solutions were prepared, each containing 0·05 mol/l phosphate buffer (pH 7·0), 0·02 mol/l $MgCl_2$, 1 μCi/ml (acetyl-1-^{14}C) choline chloride, and additional 'cold' acetylcholine in the appropriate concentration. For certain experiments at very low substrate concentrations, the (acetyl-1-^{14}C) choline chloride was simply diluted further; no 'cold' acetylcholine was added. Skin was homogenized (usually at 10 mg/ml) in 0·05 mol/l phosphate buffer (pH 7·0) containing 0·01 mol/l $MgCl_2$ and 5 mg/ml bovine serum albumin. After centrifuging to yield a clear supernatant, the residue was washed once with 2 ml buffer, and finally resuspended at the original concentration in fresh buffer.

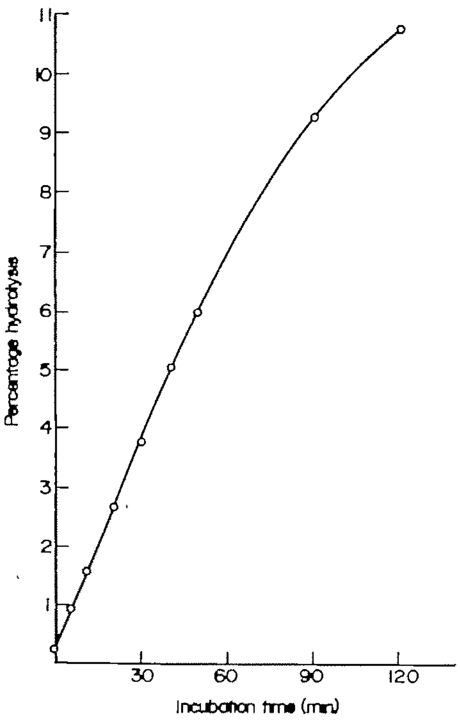

FIGURE 1. Time curve for the hydrolysis of acetylcholine (2·57 mmol/l final concentration) by a soluble preparation of mouse skin (5 mg/ml). The homogenate contained 5 mg/ml bovine serum albumin.

The reaction was initiated by the addition of 0·1 ml of the soluble or particulate skin preparation to 0·1 ml of buffered substrate solution. After incubating at 37°C for 1 h, the reaction was stopped by the addition of 0·2 ml, 0·2 N HCl and 2 ml toluene/isoamyl alcohol (5:1). The tubes were shaken vigorously, centrifuged, and the aqueous layer discarded. The organic phase was washed with a further 0·5 ml, 0·2 N HCl, and an aliquot of 1·0 ml taken for scintillation counting (Packard 'Tricarb').

A blank (in which the skin extract was replaced by buffer) was included in each experiment. After subtraction of the blank, activity was calculated as nmol (10^{-9} mol) acetylcholine hydrolysed/min/g fresh weight of skin.

Preliminary experiments

A series of assays were carried out, using mouse skin, to establish the validity of the assay technique. Variables studied included incubation time, quantity of skin, and the effect of bovine serum albumin.

The results confirmed the claims of Potter (1967) that the hydrolysis of acetylcholine by tissue extracts is linear for long periods, and that the technique described is highly reproducible even at exceedingly low enzyme concentrations. Figure 1 illustrates the time curve using an acetylcholine concentration of 2·57 mmol/l (final) and an extract prepared from 5 mg skin/ml. In the absence of bovine serum albumin a progressive loss of activity was observed, the reaction remaining linear for a few minutes only.

Kinetic studies at 'high' substrate concentrations

A total of ten soluble preparations and nine particulate suspensions of human skin was investigated. In each case the reaction velocity was measured at five acetylcholine concentrations in the range 0·54–0·074 mmol/l (final). The reciprocals of the reaction velocities were plotted against the reciprocals of the substrate concentrations, and the values of V_{max} and K_m determined by extrapolation.

TABLE 1. Kinetic data for the hydrolysis of acetylcholine in the range 0·54–0·074 mmol/l by preparations of human skin. Values of K_m are mol/l $\times 10^{-4}$, and V_{max} is expressed as nmol (10^{-9} mol)/min/g fresh weight skin

	Soluble				Particulate			
Sample no.	Non-atopic		Atopic		Non-atopic		Atopic	
	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}	K_m	V_{max}
1	4·8	40	3·2	58	4·5	22	2·2	22
2	2·5	42	3·5	48	2·9	12	4·6	30
3	2·8	45	2·2	33	2·3	17	2·6	28
4	3·5	25	2·4	32	2·5	42	3·5	47
5	3·7	28	3·0	20	2·6	62		
Mean	3·5	36	2·9	38	3·0	31	3·2	32

The results are shown in Table 1. In neither preparation is there any significant difference between the data for normal and atopic specimens.

Kinetic studies at 'low' substrate concentration

Two soluble preparations (one from normal skin, one from an atopic subject) were examined over an extended range of acetylcholine concentrations, additional measurements being made at 37, 14·4 and 7·4 μmol/l (final concentration).

The results were in both experiments identical, a biphasic Lineweaver Burk plot being obtained in each case.

These curves strongly suggest that at least two distinct enzymes utilizing acetylcholine are present in the extract. The apparent K_m of the second enzyme was $1·25 \times 10^{-5}$ mol/l (normal skin) and $0·95 \times 10^{-5}$ mol/l (atopic skin); these values lie within experimental error.

DISCUSSION

At least two carboxylic ester hydrolases present in mammalian tissue are capable of hydrolysing acetylcholine at neutral pH; these are acetylcholinesterase ('true' cholinesterase, 3.1.1.7) and cholinesterase ('pseudo' cholinesterase, 3.1.1.8) The positive identification of these is usually based on a study of their substrate specificity and of their sensitivity to specific inhibitors. Since this investigation is concerned chiefly with the ability of atopic skin to hydrolyse acetylcholine, it was considered unnecessary to distinguish the individual enzymes in this manner.

Nevertheless, our kinetic data indicate that there are indeed two soluble enzymes present in human skin, in addition to the particulate enzyme activity. The apparent K_m at 'high' substrate concentration (averaging $3{\cdot}2 \times 10^{-4}$ mol/l for all soluble preparations and $3{\cdot}1 \times 10^{-4}$ mol/l for all particulate preparations) is in close agreement with the figure of $2{\cdot}68 \times 10^{-4}$ mol/l quoted by Krupta (1964) for the 'true' cholinesterase of erythrocytes. The figures of about 10^{-5} mol/l obtained at lower substrate concentrations, on the other hand, are closer to values reported by various workers for the K_m of the 'pseudo' cholinesterase of serum. Thus, our findings support those of Magnus & Thompson (1954), who investigated the activity of minced skin towards various choline esters, and also concluded that both 'true' and 'pseudo' cholinesterase were present.

It is seen from Table 1 that there is no significant difference between normal and atopic skin in their ability to hydrolyse acetylcholine. This conclusion is in full agreement with our previous findings using β-naphthyl acetate as substrate (Cotton *et al.*, 1973). Further discussion concerning the aetiology of atopic dermatitis seems at the moment merely speculative. If indeed there is an imbalance between the sympathetic and parasympathetic pathways, there remains a real possibility that this may occur at a central rather than a peripheral level.

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Pharmacology and Treatment

A follow-up study of superficial radiotherapy for benign dermatoses: recommendations for the use of X-rays in dermatology

N.R.ROWELL

University Department of Dermatology, General Infirmary, Leeds

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SUMMARY

One hundred out of 136 patients who had received either 1600 rad or more to the face, 1500 rad or more to the hands, 1600 rad or more to the finger nails, or 1600 rad or more to the feet for benign dermatoses during the years 1930 to 1964 were examined. Four patients had developed basal cell carcinomata of the face and one had developed a squamous cell carcinoma of the hands. Atrophy of the face and keratoses and telangiectasia of the hands occurred more frequently than in controls.

It is concluded that superficial radiotherapy is a safe procedure for the treatment of benign dermatoses provided that no more than 1200 rad is given in fractionated doses to any area of skin over a lifetime. Recommendations are put forward to ensure that damage does not result from radiation.

Superficial radiotherapy for benign dermatoses has decreased in recent years but many dermatologists still find it an effective adjunct to other therapy for a limited range of conditions, such as chronic lichenified eczema, lichen simplex, resistant lesions of psoriasis, rosacea and chronic perionychia. Others have abandoned their machines completely on grounds of safety, but are they correct?

The purpose of the present survey is to establish the incidence of sequelae of superficial radiotherapy given in the Department of Dermatology of the General Infirmary, Leeds, between 1930 and 1964. Radiotherapy for cancer of the skin is not given in the Department of Dermatology, so the sequelae of such treatment has not been investigated, nor have patients epilated for ringworm of the scalp been followed up.

METHOD

Since 1930 in the Department of Dermatology at the General Infirmary at Leeds, separate record cards have been kept of all patients who have had superficial radiotherapy for benign dermatoses. These were examined in 1964 and an attempt was made to follow up all patients who had had total dosages of 2000 rad or more to the hands, 1500–1975 rad to the hands, 1600 rad or more to the face, 1600 rad or more to the nails and 1600 rad or more to the feet.

136 patients (Table 1) came into these categories and 100 patients (forty males aged between 21 and 80 years and sixty females aged between 32 and 83 years) were personally examined for the presence of carcinoma, keratoses, atrophy, telangiectasia and any other changes in the irradiated areas. In the

majority of cases radiotherapy had been given to the face for constitutional eczema, sycosis barbae or rosacea, to the hands or feet for chronic eczema or pustular psoriasis, and to the nails for chronic perionychia or psoriasis. The usual dosage schedule was 100 rad for three doses at 3- to 4-week intervals.* The quality of radiation used ranged from 60 to 90 kVp with a half-value layer of 0·75 mm Al. There was nearly always an interval of at least a year between each course. The maximum number of courses of therapy was twelve in a patient who had had 4000 rad to the hands in 20 years. Six patients had had more than 3000 rad to the hands and ten had had more than 2000 rad to the face. The longest time from the first dose of therapy to the time of follow up was 34 years. Most patients had had their final doses of radiotherapy in the early 1950s, i.e. at least 10 years before the survey.

TABLE 1. Patients attending Department of Dermatology between 1930 and 1964 with total dosage of superficial radiation given

Total radiation 1930–1964 (rad)	Patients examined	Patients not examined
2000 or more to the hands	41	36
1500–1975 to the hands	18	
1600 or more to the face	25	
1600 or more to the finger nails and terminal phalanges	11	
1600 or more to the feet	5	
Total	100	36

A similar number of control subjects, matched for age and sex, who had never had any radiation to the skin were examined for similar changes. These subjects were mainly members of staff, visitors, and patients suffering from leg ulcers or trauma.

Five subjects had had more than 6000 rad of Grenz ray for the treatment of port wine stains (capillary haemangiomata) and these were also examined.

RESULTS

Thirty-six patients were not examined for the reasons shown in Table 2. Twelve patients had died (Table 3) but there was no evidence that radiotherapy had played any part.

The sequelae observed in ninety-five of the remaining hundred patients detailed in Table 2 are shown in Tables 4–7. The remaining five patients had had from 1650 to 2350 rad to their feet but no post-radiation changes were noted. The lowest total dose of superficial radiotherapy in the present series producing the changes seen on the hands, face and neck, is shown in Table 8. Details of the patients with carcinomata are shown in Table 9 and with keratoses in Table 10. No sequelae were seen in a further three patients who had had 2300 rad to the legs, 1950 rad to the knees and 1650 rad to the groins respectively.

No sequelae were found in the five patients who had had more than 6000 rad Grenz ray. The total dosage ranged from 6400 to 12,800 rad.

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* Where pastille doses were used in the early 1930's a conversion factor of 450 rad per pastille dose has been used (Kaye & Binks, 1936).

TABLE 2. Details of patients not examined

Reason for non-examination	
Patient died	12
Patient traced but living elsewhere	6
Patient not traced	18
Total	36

TABLE 3. Details of patients who had died

Cause of death		
Known		7
Cardiac disease	4	
Respiratory infection	2	
Carcinoma of breast	1	
Unknown		5
Total		12

TABLE 4. Findings in patients who had had 2000 rad or more to the hands and in control subjects

Subjects examined	Patients (41)	Controls (41)	P
Keratoses	8	1	<0.02
Telangiectasia	8	2	<0.05
Atrophy	4	1	>0.1
Carcinoma (squamous)	1	0	>0.2

TABLE 5. Findings in patients who had had 1500–1975 rad to the hands and in control subjects

Subjects examined	Patients (18)	Controls (18)	P
Keratoses	2	1	>0.5
Telangiectasia	0	0	
Atrophy	0	1	>0.2
Carcinoma	0	0	

DISCUSSION

The only serious long-term complication of radiotherapy of the skin is carcinoma. Five patients in the present series developed a cancer. The one carcinoma on the hands was squamous in type in a patient who had had 3170 rad over a period of 23 years.

Five other patients, who had more than 3000 rad to the hands, had not developed cancer at the time of examination. The lowest dosage involved in the four patients developing cancer of the face (all basal cell) was 2090 rad. Six other patients of the present series had had more than 2000 rad to the face and none had developed a carcinoma. Table 8 shows that post-radiation changes on the whole occur at a lower dosage on the face than on the hands, suggesting either that the facial skin is more liable to be damaged by radiation or that sunlight has an additive effect in damaging the skin.

TABLE 6. Findings in patients who had had 1600 rad or more to the face and neck and in control subjects

Subjects examined	Patients (25)	Controls (25)	*P*
Telangiectases	13	15	>0.5
Atrophy	12	0	<0.001
Carcinoma (basal cell)	4	0	<0.05
Keratoses	2	0	>0.1

TABLE 7. Findings in patients who had had 1600 rad or more to the finger nails and distal phalanges and in control subjects

Subjects examined	Patients (11)	Controls (11)
Telangiectasia and atrophy (dose 2250 rad)	1	0
Other changes	0	0

TABLE 8. The lowest total dose of superficial radiotherapy in the present series to produce the changes noted

	Hands (rad)	Face and neck (rad)
Carcinoma	3170	2090
Keratoses	1800	2090
Atrophy	2175	1600
Pigmentation	2175	1600
Telangiectasia	2050	1600

Some atrophy of the skin was a feature in nearly half of those irradiated with more than 1600 rad to the face, but this was usually mild. Keratoses and telangiectasia were significantly more common in patients who had had more than 2000 rad to the hands but these features had usually not been noted by the patients. Those who developed carcinoma also showed keratoses, telangiectasia or atrophy (see Table 9), although it is known that radiation-induced carcinoma can arise in clinically normal skin as in the skin on the backs of patients irradiated for ankylosing spondylitis, and in the scalp of children irradiated for tinea capitis. Patients were also examined for pigmentation and dryness of the skin but these features were found to be difficult to assess and not of importance.

A high total dose of radiation does not necessarily result in visible sequelae. One patient who had had 2750 rad to the face had an apparently normal skin. None of the patients with keratoses had had arsenic. There was no evidence that radiation had produced any genetic effects.

It is unfortunate that eighteen patients could not be traced, but there is no reason to believe that the incidence of sequelae would be higher than in those examined. It is possible that patients irradiated with lower dosages than those reviewed in the present survey could have an increased incidence of skin cancer but we know of no cases in our department in the past 45 years.

TABLE 9. Details of the patients who had developed carcinomata

Case no.	Sex	Age at Examination	Diagnosis	Total dose (rad)	Area	Dates	Duration of follow up in years		Type of carcinoma
							Max.	Min.	
1	F	70	Rosacea	3075	Face	Between 1933 and 1942	31	22	BCC
5	F	54	Const. eczema	3170	Hands	Between 1933 and 1956	31	8	Squamous epithelioma
34	F	55	Rosacea and keratitis	2090	Face	Between 1930 and 1953	34	11	BCC
37	M	46	Const. eczema	2970	Face	Between 1936 and 1948	28	16	BCC
80	M	35	Const. eczema	2450	Face	Between 1945 and 1953	19	11	BCC

No sequelae were noted in five patients who had total exposure from Grenz ray of 6400–12,800 rad for the treatment of port wine stains.

The latent period for radiation-induced tumours varies inversely with the dose (Mole, 1972) and may be as long as 56 years (Ridley, 1962). The patients with carcinoma in the present series had been irradiated between 8 and 34 years beforehand.

Basal cell carcinomata appear to be more common than squamous cell carcinomata in association with radiotherapy for benign conditions (Ridley, 1962; Sarkany *et al.*, 1968; Albert & Omran, 1968; Kozlova, 1969), although squamous cell carcinomata are more likely on the backs of the hands. Basal cell carcinomata are particularly liable to occur in the scalps of children after radiation (Ridley,

TABLE 10. Details of the patients who had developed keratoses of the hands (no atrophy present in any patient)

Case no.	Sex	Age at examination	Diagnosis	Total dose (rad)	Dates	Duration of follow up in years		Carcinoma	Telang.
						Max.	Min.		
5	F	54	Const. eczema	3170	Between 1933 and 1956	31	8	+	+
7	F	74	Const. eczema	4000	Between 1931 and 1951	33	13	–	–
14	M	76	Const. eczema	2700	Between 1936 and 1953	28	11	–	–
18	M	53	Const. eczema	2550	Between 1945 and 1952	19	12	–	–
20	M	52	Const. eczema	3050	Between 1945 and 1953	19	11	–	–
32	F	70	Lichen simplex	1950	Between 1934 and 1950	30	14	–	–
42	F	49	Const. eczema	2900	Between 1937 and 1951	27	13	–	–
45	F	83	Const. eczema	2400	Between 1939 and 1947	25	17	–	–
58	F	77	Const. eczema	3275	Between 1940 and 1957	24	7	–	+
79	M	69	Const. eczema	1800	Between 1944 and 1963	20	1	–	–

1962) and as the result of the radiation treatment of lupus vulgaris (Pegum, 1972). Other types of tumours which have been reported after radiation include fibrosarcoma after irradiation for lupus vulgaris and hirsutes (Russell, 1959), melanoma (Traenkle, 1963) and dermal cylindroma after epilation of the scalp (Black & Jones, 1971).

Several follow-up studies of the effects of superficial radiation for benign dermatoses have been done, but none is identical with the present survey. Miescher & Böhm (1948) examined 192 patients 1–22 years after irradiation for hyperhidrosis of hands, feet and axillae. They noted that mild sequelae could occur over 1600–1800 rad. Borak, Eller & Eller, (1949) did not find any sequelae in 122 patients with hyperhidrosis who had had less than 1600 rad through 2–4 mm Al. Sulzberger, Baer & Borota, (1952) in a survey of 763 patients irradiated for benign dermatoses matched with controls, concluded that there is no evidence that fractionated doses of superficial irradiation to a total of 1000 rad or less for benign dermatoses caused any sequelae, and that when total doses more than 1000 rad were given it may be expected that 1·5% of patients would show relatively mild roentgen sequelae of only cosmetic importance.

CONCLUSIONS

Superficial radiotherapy is a safe procedure for the treatment of benign dermatoses as an adjunct to other forms of treatment provided the following recommendations are accepted.

(1) No area of skin should be subjected to more than 1200 rad in a lifetime.

(2) The dose must be fractionated. A reasonable schedule is 100 rad at 60 kVp at intervals of 3–4 weeks for three doses. At least 2 years should elapse between such courses. If further radiation is required after 900 rad has been given, Grenz ray, although less effective, should be considered as an alternative.

(3) The face is more susceptible to radiation changes and care should be taken in irradiating the face, especially in patients liable to excessive exposure to sunlight.

(4) Adequate records of all radiation should be kept.

(5) All patients should be questioned about previous radiation at other clinics.

(6) Superficial radiotherapy of the skin should only be given by staff who understand the above recommendations.

If these recommendations are carried out there is no reason to withhold a valuable form of treatment from patients suffering from certain types of skin disease, resistant to other forms of treatment.

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Behaviour therapy in dermatological practice

LOUIS H.J.BÄR AND BEN R.M.KUYPERS*

Department of Dermatology, University of Nijmegen, Javastraat 104, Nijmegen, the Netherlands

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SUMMARY

Behaviour therapy provides important psychotherapeutic possibilities for the treatment of dermatological disorders. Compulsive scratching and trichotillomania can be treated by aversive conditioning, or by the token economy technique. The treatment of blushing is described: assertive training is useful for patients with symptomatic erythema and erythrophobia. Hyperhidrosis can be treated by assertive training and systematic desensitization.

A new and important addition to the therapeutic possibilities available to the psychotherapist treating dermatological patients is the recently developed technique of *behaviour therapy*. Behaviour, or conditioning, therapy uses experimentally established principles of learning for the purpose of changing unadaptive behaviour. This unadaptive or neurotic behaviour is seen as a consequence of a faulty learning process and the aim of treatment is to weaken or eliminate them and to initiate and strengthen adaptive habits (Wolpe, 1969).

METHODS

The various techniques of behaviour therapy used in dermatological practice may be grouped into four main divisions.

Systematic desensitization

This is used mainly in neurotic disorders in which anxiety is a prominent feature. This method is described by Wolpe (1969): 'It is the breaking down of neurotic anxiety-response habits in piecemeal fashion. A psychological state inhibitory of anxiety is induced in the patient, who is then exposed to a weak anxiety-arousing stimulus. The exposure is repeated until the stimulus loses completely its ability to evoke anxiety. Then progressively "stronger" stimuli are introduced and similarly treated. This technique, which characteristically employs relaxation on the anxiety-inhibiting state, has made it possible for the first time to exert direct control over a great many neurotic habits.'

Aversion therapy

This is used in the treatment of persistent behaviour disorders, such as compulsive scratching. The method consists of giving an electric shock whenever the unadaptive habit is demonstrated or displayed.

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* Present address: St Joseph Ziekenhuis, Venlo, the Netherlands.

Operant techniques

These are also used to modify compulsive habits. Adaptive behaviour is reinforced by giving a reward; unadaptive behaviour is weakened or eliminated by being neglected or even punished.

Assertive training

This is used in patients who dare not express their emotions and who experience extreme or considerable social fear. The training consists in teaching them to behave more frankly towards the rising fear, and in that way to overcome it.

A full description of these methods and their theoretical backgrounds has been given by Wolpe (1969), Bandura (1969), Yates (1970) and Lazarus (1971).

CASE REPORTS

Compulsive scratching

Psychological investigation of patients with neurodermatitis sometimes reveals conflict situations or emotional problems. According to psychoanalytically or other psychodynamically orientated theories these conflicts have been alleged to be the *cause* of the scratching. Behaviour therapy, however, approaches these problems from another viewpoint. Walton (1960), for instance, concludes after examining a woman with neurodermatitis and compulsive scratching 'that the skin condition may well have originated because of physical considerations, though its *continuance* might have been perpetuated by psychological factors, the understanding and treatment of which could be formulated in terms of learning theory. The rewards initially associated with the continuance of the neurodermatitis had reinforced scratching until it had become a powerful compulsive habit and it was this which had therefore directly perpetuated the skin condition'. The attention that the patient got from her environment, because of her scratching, was the reinforcement which maintained the scratching. *Ignoring* this unwanted behaviour resulted in a decrease of the scratching until it stopped altogether. This reduction corresponded with a gradual improvement in the neurodermatitis which disappeared at the end of 3 months and did not return within 4 years. In conditioning terms, stopping the reinforcement of the neurotic behaviour resulted in extinction of the undesired and unadaptive habit.

It is also possible, though, to *reward* the desired behaviour (non-scratching). This form of *operant conditioning* also appears to be very useful in the treatment of compulsive behaviour and may be used in combination with ignoring the existing behaviour. In principle the method consists of rewarding the patient for not having scratched for a certain period of time. Because it is in practice very difficult to give a valid reward every time, one can use secondary reinforcers or tokens. With this method, the *token economy* system, the patient receives a token after a short fixed interval, for instance an hour (dependent on the schedule or reinforcement). If after a longer period he has still not scratched, for instance a day, he may hand in a fixed number of tokens (secondary reinforcement) in return for a very attractive reward (primary reinforcement). With the help of this method compulsive scratching, especially in *children*, can be unlearned within a relatively short time.

Allen & Harris (1966) described the treatment by such a method of a girl aged 5 with excessive scratching. These authors are of the opinion, after a 3 year study, that 'problem behaviour of normal children was controlled by its immediate consequence, the attention of adults'. It is important then 'to teach parents to modify their own attending behaviour in ways that might then resolve their child's problem'. The authors persuaded the parents to ignore scratching and to reinforce desirable behaviour (non-scratching) by tokens. They report that at the end of 6 weeks the child's face and body were clear of all scabs and sores. As the scratching decreased, the mother was instructed in appropriate techniques for reducing the reinforcement schedule. Four months later the scratching behaviour had not recurred. Our treatment procedure and results correspond with those described by Allen & Harris.

Case 1. A girl aged 6 with severe neurodermatitis of the vulva had scratched herself day and night for 3 years. After the disappearance of the physical cause (Oxyuris), the scratching persisted. Two periods in hospital had no success. The girl was a hyperactive, restless child who demanded much attention from the environment. The mother was a rather rationalistic woman, who frequently discussed the scratching with the patient and who had tried many remedies for this behaviour. We hypothesized that the scratching behaviour was a form of attention seeking by the child, in which this unadaptive behaviour was at the same time reinforced by the attention received by it from the environment. The treatment plan involved giving the child the necessary positive attention, not as a result of scratching, but rather as a reward for not scratching. The mother was given instructions to ignore completely all scratching behaviour and to give attention to the child at the moments of non-scratching. Each hour the child received a token if scratching had been absent during that period. During schooltime the programme was continued in the same way. Every evening the girl could exchange the tokens of the day for a sweet. For the night period she always had a favourite and valued toy to look forward to. After 5 weeks she scratched distinctly less during the night and after 13 weeks the programme could be stopped (Fig. 1). 18 months' follow-up showed no relapses. In conditioning terms, stopping the reinforcement of the neurotic behaviour and reinforcing the desired behaviour resulted in extinction of the undesired and unadaptive habit.

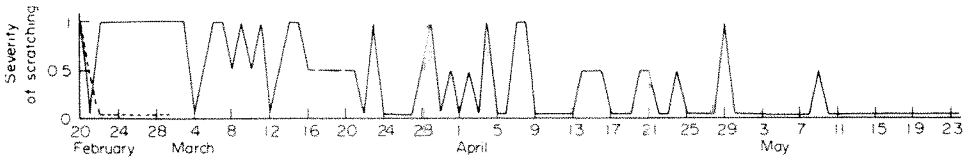

FIGURE 1. Course of scratching in a case of neurodermatitis treated by a token economy procedure. Scratching: 1, heavy; 0·5, slight; 0, nil; —, night; - - - -, day.

Sometimes the psychologist can find no psychological or social problems which maintain the compulsive behaviour, yet the patient scratches and often the neurodermatitis spreads further. A close analysis will then often show that, here too, the scratching has had a physical cause, which has now disappeared, whereas the scratching persists. The positive reinforcement which perpetuates the scratching appears thus to be the abolition of the unbearable itch (tension) and in consequence tension reduction. By scratching, the itch diminishes, which is an enjoyable experience, which again reinforces the urge to scratch. In this way a vicious circle is set up. In such a case it is reasonable to break the vicious circle by stopping the act of scratching from being enjoyable, that is to make it unpleasant, and thus to evoke in the patient an aversion against scratching. Such a method is described by Ratliff & Stein (1968). They treated a patient with severe neurodermatitis. The treatment plan involved *aversive conditioning* procedures. The authors gave the subject an electric shock (1 mA) whenever he scratched any part of his body. The shock was terminated immediately after he stopped scratching and said aloud, 'Don't scratch'. An alternative, but complementary, treatment approach was adopted by the authors in the hope of establishing more permanent and general therapeutic effects. This approach involved training the patient in progressive relaxation with instructions to apply this technique each time he experienced the 'urge to scratch'. It was hypothesized that relaxation should serve to compete with the unpleasant sensations resulting from not scratching, and either to maintain or facilitate the acquisition of alternative responses incompatible with scratching. After about 5 weeks under this treatment the maladaptive scratching had been completely eliminated and did not return within the 6 months follow-up. Our own method of aversion therapy is a variation of this.

Case 2. A man aged 33 suffered from severe lichen simplex on the scrotum, thighs and ankles, which had been present for 4 years and for which he had been admitted to the clinic three times, without any lasting result. According to the patient the scratching had originally developed as a result of pruritus ani due to terminal ileitis. From the psychological examination the patient emerged as a rigid, tense personality, who could only with great difficulty cope with interpersonal relationships and could not express his feelings adequately. Because of a lack of motivation and insight he did not appear to be accessible to verbal psychotherapy. Therefore we set up the following aversion procedure. The patient was seen once a day for a 20 min session. He was instructed to bring his hand to his scrotum, upper leg or ankle to scratch at a sign from the therapist. This sign was given every 15 s*. At the moment his hand reached one of these sites, he received an unpleasant, though not painful, electric shock via electrodes on the fingers of the moving hand. He then had to draw back his hand and say aloud, 'Don't scratch'. In each session he received seventy-six shocks. He was also trained in relaxation exercises. After 19 days of treatment the scratching had disappeared completely and there was a consequent improvement in the skin disorder (Fig. 2). Follow-up after 13 months showed that he had suffered three short, severe attacks of itching during which he could not refrain from scratching. After the end of such an attack there was no further scratching. In conditioning terms, punishing the neurotic behaviour resulted in deconditioning the undesired and maladaptive habit.

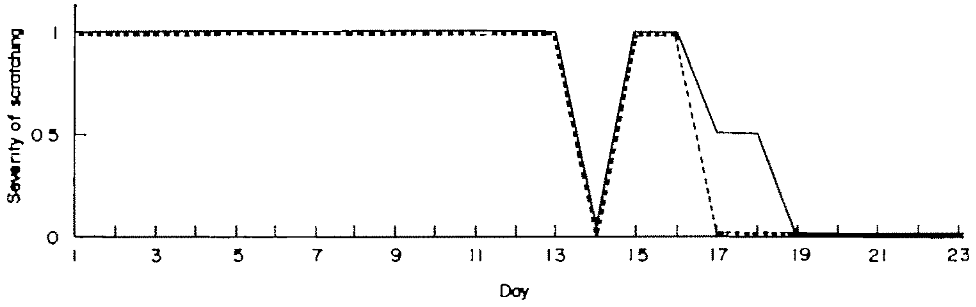

FIGURE 2. Course of scratching in a case of neurodermatitis treated by shock-aversion therapy. Scratching: 1, heavy; 0·5, slight; 0, nil; —, night; - - - -, day.

Trichotillomania

Other forms of compulsive behaviour are the plucking of hairs from the head and eyebrows (trichotillomania), the production of dermatological artefacts and self-mutilation of the nails. Here too it is important to carry out an accurate psychosocial investigation. As with compulsive scratching it may be that existing emotional problems are not the cause of the undesired behaviour, but can be understood as a consequence of, or as a condition for, the maintainance of this behaviour which has begun to have an autonomous existence.

Taylor (1963) describes the treatment of a case of obsessive, compulsive eyebrow plucking and states that 'since the emotional concomitants of the behaviour are a consequence of social criticism, which in turn is a consequence of the compulsive action, it is evident that any attempt to deal directly with the emotion must fail', and 'our theory leads to the conclusion that the primary habit is likely to be the simplest component of the whole behavioural complex, and therefore most amenable to treatment'. The patient described was a woman aged 40, who had been plucking her eyebrows compulsively for 31 years. She was instructed to stop at its commencement any movement of her hands to her forehead for the purpose of plucking. Cure was essentially complete in 10 days and 3 months later the eyebrows were fully grown. The rationale suggested by the author for this method was that 'every time the impulse arose the patient was aware of it, not merely as an impulse to raise the hand to the forehead but as an impulse to pluck; and by preventing the initial response from progressing beyond the first millimetre she was in fact breaking down the whole response sequence'.

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* It appeared from further experience that administering the shock every 5 s was equally effective.

There are patients, however, in whom the plucking of hairs is more or less unconscious. An instruction as given by Taylor is not sufficient in such cases. These patients have to be made aware that they are committing the unwanted activity. With the help of aversion therapy, as is described for scratching, the plucking or pulling appears to be rather rapidly brought under control. In the process of becoming more conscious, cognitive factors certainly play some significant role.

Case 3. A boy aged 14 had shown symptoms of trichotillomania for 1½ years. He had developed the habit of pulling out his hair, especially whilst he was reading or studying. A psychological investigation gave no indication of emotional problems. Pulling of his hair turned out to have a sensual significance for him. He reported that he knew that he pulled out his hair, but that he was not aware when he was actually doing it. The treatment consisted in breaking down the vicious circle by means of aversion therapy. In a period of 14 days he was able to refrain from this habit. He felt himself to have gained esteem from the people in his environment and he reported increased self-confidence. 2 months later there was some sporadic hair pulling, but after several 'booster' sessions these residual symptoms totally disappeared. One of the disadvantages of aversion therapy is that it cannot always prevent a slight relapse into the old habits which can become very rewarding for the unwanted behaviour. Therefore we advise some 'booster' sessions if necessary.

Blushing

Not infrequently the dermatologist is confronted with patients who complain about chronic blushing. Psychological examination of these patients often shows that they are timid and inhibited in social contacts and situations. They dare not express their feelings, for fear of disapproval from others, so that they bottle up their emotions and begin to feel inferior. In social situations these patients react with autonomic vasomotor responses, including those in the face (symptomatic erythema), as an expression of their inhibited but conscious fears.

Based upon their social fear, which expresses itself in symptomatic erythema, a second form of fear often develops, namely the fear of becoming red (erythrophobia). Not infrequently the same kind of erythrophobia develops on the basis of an existing redness as in eczema or rosacea. So here there is a second conditioning process in which the erythrophobia becomes an emotional response which, according to the classical conditioning paradigm, is elicited by the threatening situation and the symptomatic erythema connected to it. Gibbs (1965) states: 'The blushing exacerbates the anxiety in a secondary way since the symptom itself embarrasses the patient'. Since in these cases the inhibition of the ability to express emotions is the basic problem, the therapy will have to be directed towards learning to experience, accept and to express the inhibited emotions; such as anger, discontent, aggression, friendliness or affection, towards other people. Instead of escaping from the situation (e.g. by not expressing emotions) one trains the patient to behave more frankly in the face of the rising fear and thus to overcome it.

This method is called *assertive training* (Salter, 1950; Wolpe & Lazarus, 1966; Lazarus, 1971) and is based on Wolpe's theory of reciprocal inhibition (Wolpe, 1958). Assertive methods of training include amongst others: learning to express spontaneous feelings; learning to express one's own opinion clearly; learning to accept acknowledgments and praise; learning to cope with certain threatening situations, with the help of acting or role playing (behavioural rehearsal), where the therapist reinforces new, appropriate responses by praising them systematically. The theoretical background is that this form of behaviour will be strengthened in daily life by its positive consequences.

Salter, who was the designer of assertive training, described a case of chronic blushing in a patient with emotional inhibition phenomena and social fear (Salter, 1950, case 10). It is remarkable that, before the patient was instructed in his 'six techniques for increasing excitation' the author instructed him to try to blush as much as possible in all kinds of situations and places. Within a week the patient appeared to have completely brought the involuntary blushing under voluntary control. Gibbs (1965) describes the treatment of a case of symptomatic erythema. The subject had been seriously troubled by excessive blushing in social situations for the previous 7 years. He was a man who constantly smiled, even under strong provocation. His embarrassment and blushing were greatest in personal contacts with his superiors in the hierarchy, with whom he never disagreed. He frequently felt an impulse to be aggressive, but always inhibited its expression. Gibbs postulated that the symptoms were a learned response to anxiety. Treatment involved training in assertiveness. Considerable reduction in the frequency of blushing, together with improved social competence, were the results; the improvement was maintained over a follow-up period of 6 months.

Case 4. A student aged 22 with chronic constitutional eczema had also suffered from a symptomatic erythema for 4 years. The patient was descended from a narrow-minded, petit-bourgeois milieu, which he had outgrown by his studies and in which he felt out of place. The chronic blushing developed when he started studying in a foreign city. He was tormented by deep feelings of inferiority and uncertainty, which, strengthened by his existing skin disorder, handicapped normal social contacts until he retreated into almost complete solitude. With the help of the school doctor the worst phenomena of social fear were conquered, but after that he reacted to less subjective social threats by blushing with increased redness of his already red face, as a result of which the fear of blushing increased systematically. We attempted to break through this vicious circle by means of assertive training. The patient was advised to try to blush in as many situations as possible. After 5 days the blushing did not appear anymore. The feelings which assailed the patient in social situations, but which he tried to 'suppress' spasmodically, were discussed and were thus experienced and accepted. During this process the therapist handled his approving remarks as operant reinforcers. Eventually the patient was taught to express his emotions, and also shown how he could make headway against socially threatening situations and thus overcome his fear. The social anxiety and fear of blushing disappeared rather rapidly and made room for a gradually strengthening feeling of self-confidence and skill in coping with interpersonal situations. 6 months later a check up showed not only no recurrence or symptom substitution, but also a continuously increasing ability to express himself adequately in social situations.

Although social fear is one of the most important characteristics of chronic blushing* this is not always determined by, or based upon, a lack of assertiveness. With accurate examination the existence of a phobia can sometimes be revealed in regard to certain specific aspects or objects in the environment. As Wolpe (1969) states: 'Frequently fear and avoidance of social situations turn out to be based in fear of criticism or of rejection; or the fear may be a function of the mere physical presence of people, varying with the number to whom the patient is exposed'. In these cases another therapeutic strategy must be applied to decondition the phobia; as for instance *systematic desensitization.*

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* In many patients with urticaria there are not infrequently social fear, inhibition of feelings and a lack of assertiveness. In these cases assertive training can often be successfully used.

Hyperhidrosis

Certain psychic reactions, like terror, fear, fury and strong concentration, are attended by increased sweating (Kuno, 1956). The palms and soles sweat more than normally in difficult life-situations and during psychic stress. Many people react even to small emotional stimuli with hyperhidrosis. This can give rise to secondary skin damage. Possibly a congenital form of hyperhidrosis also exists. This disorder can develop too as a result of contact hypersensitivity to certain substances.

Many patients with hyperhidrosis are afraid and not able to express their emotions. They can moreover become involved in a sort of vicious circle; the excessive perspiration or the fear of starting to sweat is sufficient to strengthen the fear and with that the hyperhidrosis (sudophobia). Some patients are not aware of this underlying fear, i.e. they only realize that they become frightened in a social situation, because someone may find out that they have wet hands. Psychological examination of these patients shows hypersensitive, restless, tense or frightened people. One can treat a number of these patients with sedatives and psychotherapeutic treatments. In patients who have repressed their fear very deeply the motivation for psychotherapeutic treatment is often absent. It is therapeutically sensible in these cases to provide help in breaking through the fear of social situations. *Assertive training* can produce good results here; i.e. the patient is taught to conquer fear reactions in interpersonal situations. *Systematic desensitization* can also be a good adjunct to the treatment.

Case 5. A girl aged 19 attended the dermatology out-patient clinic because of her severe palmar hyperhidrosis, present for some years and interfering with her work. She appeared to be in particularly great need of personal interest. As a child she felt that she was rapidly excluded by other people. She was also brought up with the idea that she had to do everything very well. She gradually developed strong feelings of insecurity when the environment did not regularly express positive appreciation of her. Moreover, she was not able to express her 'negative' feelings. Her skin disorder became an extra reinforcement for her insecurity. The mere thought of having to shake hands with somebody produced an outburst of sweating. So it is possible that the sweating had become a conditioned reaction of fear for threatening stimili (social situations). The normal sweat reaction to psychogenic stimuli, which was practically continuously active because of her insecurity and fear, was further reinforced by her fear of this reaction.

Through assertive training and systematic desensitization it became possible for the patient to feel more secure in social situations, to which her skin disorder reacted positively. Although the sweat gland activity was not completely 'normal' even after some weeks, the patient was content with the improvement. A follow-up after 12 months showed no return to her old behaviour pattern.

We are now developing techniques in which, along with the above mentioned therapeutic methods, an attempt is made to condition instrumentally the sweat gland activity itself (Kuypers & Cotton, 1972).

Phobias

In those cases characterized by a fear of certain objects, situations or diseases, like syphilophobia or cancerophobia, perhaps *systematic desensitization* could be used. Case studies have not yet been published and our own experience with behaviour therapy of patients of this type is at the moment insufficient for a definite conclusion.

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Case Reports

Oral mucosal neuroma–medullary thyroid carcinoma syndrome

D.MURRAY WALKER

Department of Oral Medicine and Oral Pathology,
Welsh National School of Medicine, Cardiff

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SUMMARY

A case history of the multiple mucosal neuroma–medullary thyroid carcinoma syndrome is reported. The diagnosis first suggested by neuromas confined to the oral cavity, led to evidence of clinically unsuspected residual thyroid tumour in a patient who had undergone total thyroidectomy. The opportunity for the clinician when confronted with such oral neuromas to initiate a search for a thyroid carcinoma is emphasized, since neuromas may precede this potentially fatal thyroid tumour by as much as 10 years.

The association of multiple mucosal true neuromas and endocrine tumours such as medullary thyroid carcinoma and phaeochromocytoma was first noted by Williams & Pollock (1966).

CASE REPORT

J.S., a male gardener aged 21 was referred by his general medical practitioner to a consultant general surgeon, Mr D. W. Thomas, at Aberdare. When J.S. was 14, his mother had first noticed a hard swelling on the right side of his neck.

On examination there was a hard nodule in the right lobe of the thyroid gland. Bilateral subtotal lobectomies of the thyroid gland were performed and the nodule reported upon as 'papillary' thyroid carcinoma. Consequently a total thyroidectomy was performed by Mr J. S. H. Wade at the Cardiff Royal Infirmary. While undergoing convalescence he presented at the University Hospital of Wales Dental Hospital with toothache and a number of carious teeth were dressed. Multiple oral mucosal nodules were noted but their significance was not then appreciated nor, in fact, on several subsequent occasions when seen by different members of the dental hospital staff during 1969 and 1970, until 21 October 1971 when study of the paper published by Bartlett *et al.* (1971) first suggested that perhaps this patient was suffering from the oral mucosal neuroma–medullary thyroid carcinoma syndrome. The patient was now examined in the light of this diagnosis and certain new features were observed, and others reassessed.

The patient was a fit looking man, with no signs of thyroid tumour recurrence 2 years after thyroidectomy. The upper and lower lips were full and actually protruberant. The nodules on the dorsum of the tongue were firm, sessile, up to 5 mm in diameter and covered by non-papillated mucosa with small vessels clearly coursing over their surface (Fig. 1). A soft 8 mm diameter polyp was present behind each commissure. On general examination, the positive physical findings were a large pigmented naevus on the right thigh and both feet had the pes cavus deformity. His blood pressure was found to be normal on several occasions. There was no other relevant past medical history but his paternal grandfather had had a 'toxic goitre'. All these features supported a provisional clinical diagnosis of the oral mucosal neuroma–medullary thyroid carcinoma syndrome.

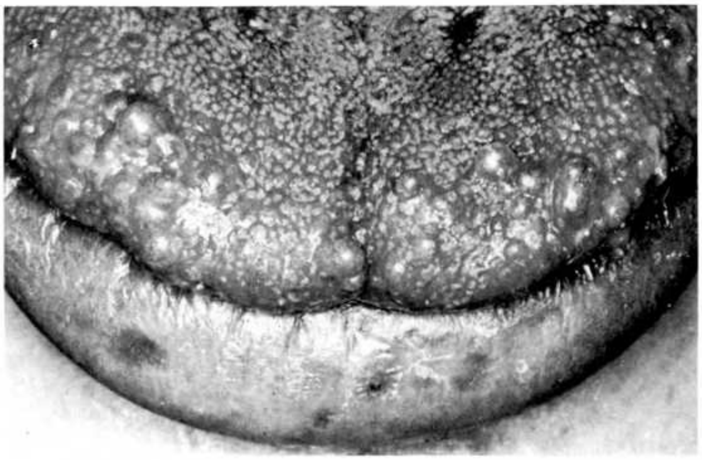


FIGURE 1. Multiple neuromas on anterior dorsum of tongue.

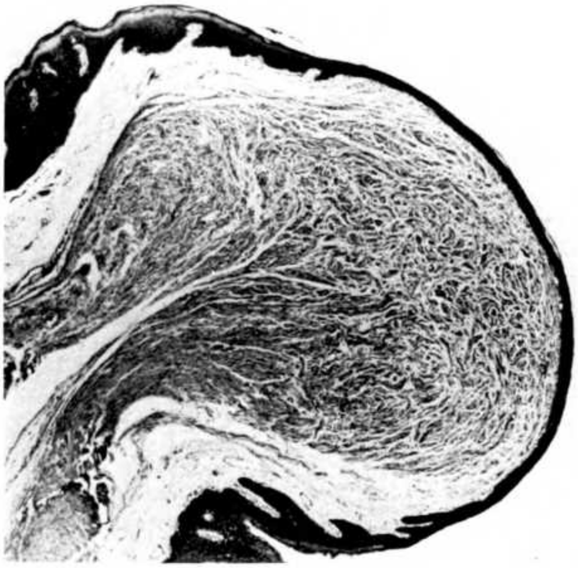
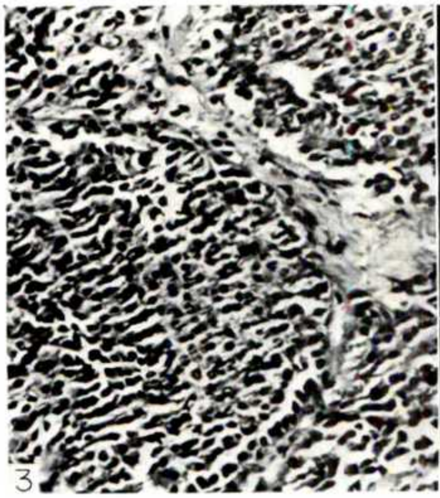


FIGURE 2. Section of one of the nodules from dorsum of tongue. Histology confirms them to be true neuromas, consisting mainly of intertwined bundles of nerve fibres (silver, × 7).

Histological examination of the mucosal nodules on the dorsum of the tongue proved them to be true neuromas consisting mainly of intertwined bundles of nerve fibres (Fig. 2).

Specimens of the original thyroid tumour were then re-examined and histologically the 'papillary' tumour was, in fact, a medullary thyroid carcinoma consisting of sheet-like masses of rounded cells of medium size (Fig. 3) separated into islands of variable size by the irregular trabecular stroma which contained identifiable amyloid (Fig. 4).



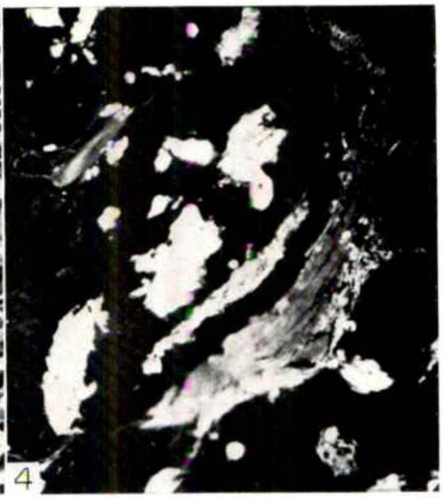


FIGURE 3. Histological appearance of the medullary thyroid carcinoma, with typical sheets of rounded cells, separated into islands by stroma (H & E, × 40).

FIGURE 4. Section of the medullary thyroid carcinoma; the amyloid material fluoresces under ultra violet light (Thioflavein-T, × 28).

Special investigations

Routine haematological and biochemical investigations were normal. However, a random serum calcitonin estimation was found to be >100 ng/ml, significantly above the normal adult value of up to 200 pg/ml, strong evidence for the presence of previously unsuspected residual thyroid tumour. Tests for urinary catecholamine derivatives were raised above normal but not diagnostic (VMA 9·2 mg/24 h, total metadrenalines (MA) 0·94 mg/24 h).

DISCUSSION

In this syndrome, true neuromas of the mucous membranes, notably of the oral mucosa and conjunctiva, are found together with endocrine tumours such as medullary thyroid carcinoma and phaeochromocytoma. From a review by Gorlin *et al.* (1968) other endocrine disorders may be found, such as parathyroid tumours, diabetes mellitus, Cushing's syndrome, together with skeletal defects such as pes cavus (as in this patient). Café-au-lait spots occur, of which J.S.'s pigmented naevus may be a variant. Intestinal ganglioneuromatosis and diverticulosis of the colon, and other abnormalities may be associated features.

Of the attempts to explain these diverse and apparently unrelated disorders found in this syndrome on a common aetiological basis, that by Schimke *et al.* (1968) seems most satisfactory. They postulate that the syndrome is caused by a generalized disorder of neural crest development. In favour of this theory, the parafollicular calcitonin-secreting 'C' cells of the thyroid from which medullary thyroid carcinoma arises, the adrenal medulla which gives rise to the phaeochromocytoma, the pigmented naevus cells, and the neuromas are all neural crest derivatives.

Thus one of the implications of the change in diagnosis was to make a search necessary for a catecholamine-secreting phaeochromocytoma. Although the patient has no signs or symptoms of such a tumour, urine tests for catecholamine were raised but not diagnostic.

Medullary thyroid carcinoma

Medullary carcinoma of the thyroid tends to occur at a younger age than other types of thyroid carcinoma. It is distinguished from these other tumours furthermore by the content of amyloid. Other substances such as calcitonin (Melvin & Tashjian, 1968), histaminase (Baylin *et al.*, 1970) as well as prostaglandin (Williams, Karim & Sandler, 1968) and serotonin are produced in addition. Measurement of the serum calcitonin, preferably under basal conditions, has been found to be a reliable test for the early detection of both the localized medullary thyroid tumour and of residual tumour after surgery. Thus, in the patient J.S., the raised serum calcitonin is evidence of residual tumour.

Prognosis

One important result of the diagnosis of the syndrome was to alter the prognosis of the patient. Medullary carcinoma of the thyroid has generally been held in the past to have a worse prognosis compared with the papillary variety, being intermediate in this respect between papillary and the solid anaplastic tumour.

Diagnosis and treatment

Furthermore, as regards diagnosis and treatment, medullary thyroid carcinomas do not take up radioactive iodine (^{131}I) in a tracer dose and do not respond to radiotherapy (Davies, 1967).

Genetic counselling

Medullary thyroid carcinoma and its associated conditions may be sporadic (85–90%) or familial (Keynes & Till, 1971). In spite of the absence of any family history of thyroid carcinoma or mucosal nodules it was felt that J.S. was more likely to be a patient with the genetically determined thyroid carcinoma. These familial tumours usually present before the age of 30 years (J.S. was 14 years of age) whereas the sporadic group mainly occur between 50–70 years of age and are less commonly bilateral. Secondly, mucosal neuromas mainly occur with the genetic group of tumours. The mode of inheritance in the familial group is known to be that of an autosomal dominant with almost complete penetrance (Williams, 1970). Thus, in the case of J.S., especially as he is unaware of his diagnosis, careful thought will have to be given to the question as to whether genetic counselling should be given and its nature. Furthermore, it is desirable to examine the eyes, mouth and thyroid glands of the relatives of the patient. The serum calcitonin has also been suggested as a means of screening relatives of patients with medullary thyroid carcinoma.

CONCLUSIONS

In conclusion, the oral manifestations of this syndrome, namely, multiple true mucosal neuromas arranged in a pathognomonic distribution in the mouth appear at birth or in the first few years of life. Thus, these oral signs may precede clinical symptoms of a medullary thyroid carcinoma by up to 10 years. In Barlett *et al.*'s familial case (1971), the 4-year-old grandson of the original patient with this syndrome was found to have these oral mucosal neuromas only and no other abnormalities. On this basis a prophylactic thyroidectomy was confidently performed. The thyroid showed an early medullary thyroid carcinoma.

Thus, when confronted with oral mucosal neuromas, the clinician in diagnosing this syndrome may have the rare opportunity of initiating the prompt treatment of a potentially fatal thyroid malignancy at an early and even clinically undetectable stage.

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Subcorneal pustules in erythema multiforme

IAN B. SNEDDON

Rupert Hallam Department of Dermatology, United Sheffield Hospitals

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SUMMARY

A man aged 24, suffering from Stevens Johnson syndrome precipitated by an upper respiratory infection, developed a skin eruption which consisted of sterile pustules. Histological examination showed subcorneal pustules containing polymorphonuclear leukocytes and no abnormality of the basal part of the epidermis, an appearance indistinguishable from subcorneal pustular dermatosis. The eruption cleared rapidly and did not recur. It is emphasized that the histological appearance of a subcorneal pustule does not necessarily indicate a diagnosis of subcorneal pustular dermatosis (Sneddon & Wilkinson, 1956).

Subcorneal blisters containing polymorphonuclear leukocytes occur in a number of disorders which include impetigo, subcorneal pustular dermatosis (Sneddon & Wilkinson, 1956), pemphigus foliaceus and pustular psoriasis (Wilkinson, 1969). They have not been described in acute erythema multiforme in which the blister is situated subepidermally and is a result of necrosis of the lower layers of the epidermis and oedema in the dermis (Caulfield & Wilgram, 1962). In view of the tendency to diagnose any subcorneal pustular eruption on the basis of the pathological change as subcorneal pustular dermatosis, the following case report is of interest.

CASE REPORT

A.K., a waterworks labourer aged 24, was admitted to hospital on 14 January 1972. 4 days previously he had developed aching in the limbs, malaise and a cough with sputum. He was prescribed Codis and Benylin expectorant by his family doctor and within 24 h his mouth and eyes became painful and an irritable eruption developed on his limbs with a few scattered lesions on his trunk. His mouth became so painful he could not eat and his urethral orifice also became inflamed. Ampicillin was given for 24 h but as he did not improve hospital admission was arranged.

On admission his temperature was 38·5 °C and he showed an acute conjunctivitis with photophobia. The oral mucosa was necrotic and the necrosis extended to the red margin of the lips. The urethral orifice was ulcerated and there was a serous urethral discharge. An eruption of erythematous discs with central bullae was scattered over the arms, forearms, thighs and legs, and there were sparse lesions also on his back. The lesions were target in type and varied from 2 to 3 mm to 1 cm in diameter. An unusual and striking feature was that the bullae contained pus and when he sat up in bed the bullae on the arms and trunk showed pus in the lower dependent part with clear supernatant fluid above, a change seen frequently in subcorneal pustular dermatosis.

A clinical diagnosis of erythema multiforme of the Stevens Johnson type was made, but in view of the unusual pustular eruption a skin biopsy was carried out on a small intact bulla on the right arm. Investigations were as follows—blood sugar 102 mg/100 ml, haemoglobin 16·6 g/100 ml, WBC 7000/mm^3, ESR Westergren 80 mm in 1 h. A gram film from the blister showed only debris with no organisms and on culture was sterile. The urine showed a trace of albumin but was sterile. Radiograph of the chest demonstrated some consolidation at the base of the right upper lobe. Swab from the penis showed pus cells and grew a culture of coliforms, *Staphylococcus albus* and enterococcus. Anti-streptolysin O titre 200 units. Serum transaminases were within normal levels. Wasserman and Kahn and Reiter precipitation tests were negative. Immunoglobulins showed slightly raised IgM 204 (normal 47–170) compatible with a virus disease. Cold agglutinins positive to a titre of 1 in 32. Sputum showed no mycoplasma on culture. Epithelial antibody estimations were not carried out. Histological examination showed that in the epidermis there was a subcorneal pustule which contained numerous polymorphonuclear leukocytes (Fig. 1).

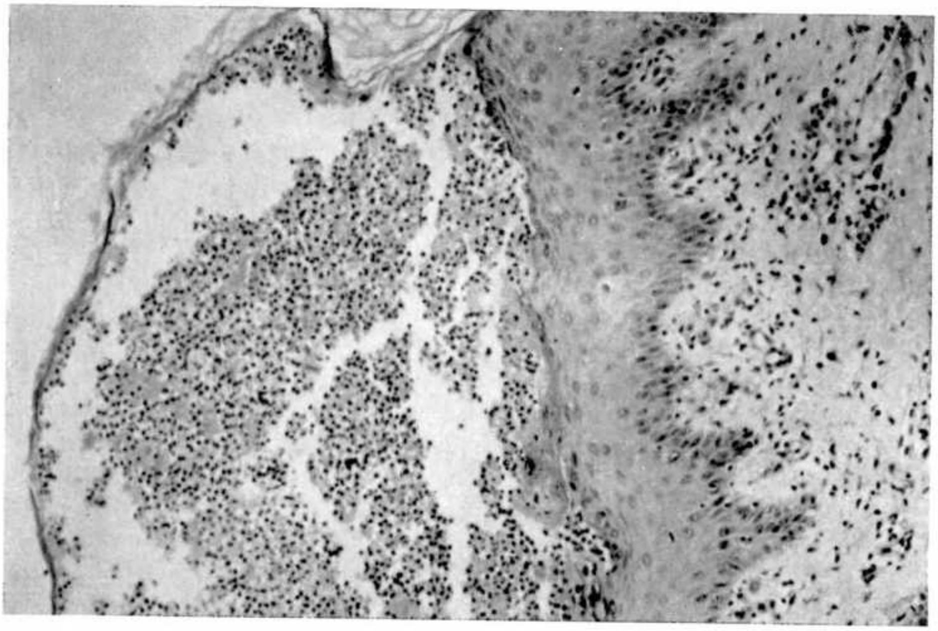

FIGURE 1. Subcorneal pustule containing polymorphonuclear leukocytes. Note the absence of intracellular oedema of the lower layers of the epidermis and of any necrosis of the basal cell layer, the usual change found in erythema multiforme (× 100).

The lower part of the epidermis was entirely normal and there was no evidence of intercellular oedema, inflammatory cells or necrosis of the basal cells. Slight dilatation of the blood vessels and a mild perivascular infiltration of lymphocytes was present in the upper dermis. In view of the severity of the mucous membrane lesions he was maintained on Ampicillin and given prednisolone phosphate 30 mg daily for 6 days and this was then discontinued gradually in the next 4 days. The eruption speedily subsided and was clear 13 days after admission at which time a radiograph of his chest showed the lung fields to be also clear.

DISCUSSION

On clinical grounds and on the results of the investigations it appears that the patient did have an erythema multiforme eruption of the Stevens Johnson type associated with an upper respiratory infection and a mild pneumonic process. The immunoglobulin and cold agglutinin results suggest that this was probably of a mycoplasmal or viral origin, though confirmation was not obtained. His symptoms developed before he was given any drugs and it would seem likely that the condition was triggered off by infection rather than by drug sensitivity.

It is difficult, however, to explain the clinical and histological findings which differ so much from the usual case of erythema multiforme. Even clinically it was apparent that the bullae were somewhat unusual. It is not usual to have pus which separates into two layers in the bulla of erythema multiforme; histologically there was a subcorneal pustule filled with polymorphonuclear leukocytes. The fluid was sterile so one can exclude secondary infection with pyogenic bacteria and, moreover, the distribution of the eruption was that of erythema multiforme. However, the microscopical changes were not those of erythema multiforme, which usually shows intense oedema in the dermis and necrosis of the lower layers of the epidermis producing a subepidermal blister. In a recent electron microscopical study of the bullae of severe erythema multiforme, Prutkin & Fellner (1971) demonstrated that there was, as well as an inflammatory reaction in the dermis, striking epidermal oedema in the early lesions with loss of architecture of the basal cells, an entirely different picture from that shown in the present case. The course of the disease, particularly the speedy disappearance of the eruption, can exclude the more chronic subcorneal pustular eruption originally described by Wilkinson and myself. A personal communication from Donaldson (1972) who has had a very similar case which followed an acute infection and which also showed the histological features of subcorneal pustular eruption suggests that this case is by no means unique.

Drugs

Codis, soluble aspirin and codeine. Benylin expectorant, diphenhydramine hydrochloride in a flavoured syrup.

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Chronic benign familial pemphigus

INDUCTION OF LESIONS BY *HERPESVIRUS HOMINIS*

BARBARA LEPPARD*, T.J.DELANEY AND K.V.SANDERSON

Department of Dermatology, St George's Hospital, London, S.W.1

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SUMMARY

A case of chronic benign familial pemphigus (CBFP) is described in which a severe exacerbation was associated with infection with *Herpesvirus hominis*. This occurrence, which has a bearing on treatment, may not be as uncommon as the absence of previous reports would suggest.

The two original descriptions of chronic benign familial pemphigus agree on the main features of the disorder. Hailey & Hailey (1939) emphasized the familial nature, the tendency to recurrent attacks over long periods from early adult life onwards and the absence of constitutional signs and symptoms. In choosing a title for their new disease they were influenced by the histological resemblance of the blisters to pemphigus. Ayers & Anderson (1939) noted, in addition, that fresh unopened vesicles could yield staphylococci or candida, and they were so impressed by the resemblance of the early lesions to herpes simplex that they proposed the name 'recurrent herpetiform dermatitis repens'. Several reports since then have suggested that the recurrences may be due, in part at least, to infection of the affected areas by *Staphylococcus pyogenes* (Lowenthal, 1959) or *Candida albicans* (Burns *et al.*, 1967).

Although Ayres and Anderson suggested that the onset of recurrences might be related to herpes simplex we can find no reference to herpes virus being found, or indeed being looked for in CBFP. It seems worth while therefore to record a case in which a relapse of unusual severity was shown to be due to *H. hominis* and to discuss the implications of this finding in the treatment of the disease.

CASE REPORT

A 38-year-old white Turkish Cypriot man† was first seen in May 1972 with a 12-year history of perianal irritation. He said that his father and two of his father's sisters, living in Cyprus, had had similar perianal irritation but had not been diagnosed as having CBFP. His irritation was always worse in the summer, almost disappearing in the winter. 1 week before he was seen, he developed a burning sensation of the groins, scrotum and perianal area with blistering of the skin. On the day of presentation severe pain had developed in the area, a symptom he had not previously experienced.

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* Present address: St John's Hospital for Diseases of the Skin, London, W.C.2.

† Presented at the meeting of St John's Hospital Dermatological Society 29 June 1972.

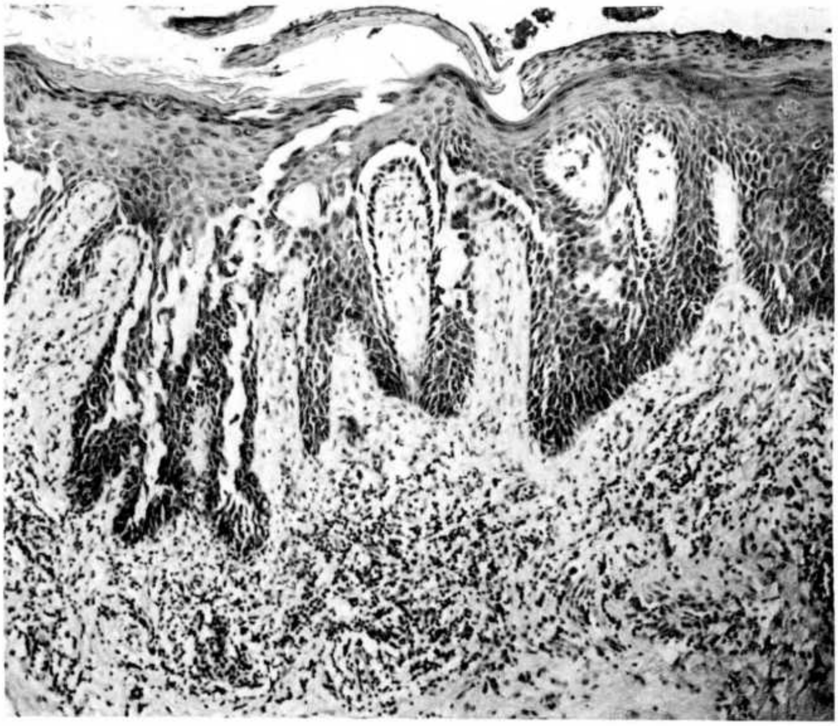

FIGURE 1. Histology of scrotal lesion (× 160).

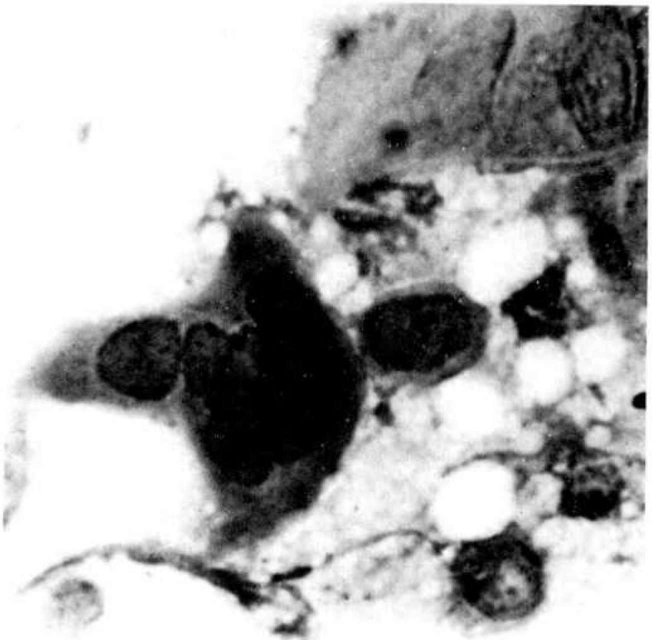

FIGURE 2. Tzanck smear on 7th day of attack. *Two* multinucleate giant cells, and several acantholytic cells are seen (× 800).

Local corticosteroid creams, Nystatin and chlortetracycline had not helped him in the past or on this occasion. He had had recurrent herpes simplex infections of the lips for many years, but had not any recent lesions. His wife had never had 'cold sores'.

Examination showed maceration and fissuring of the skin of the scrotum, the upper and inner thighs and the perianal area. There were also small bullae in some areas, grouped like those of herpes simplex.

Biopsy confirmed the clinical diagnosis of CBFP. The epidermis showed acantholysis with intra-epidermal bullae (Fig. 1). No giant cells or inclusion bodies were seen. Tzanck smears taken from the scrotum when he was first seen showed groups of acantholytic cells and several ballooned or multinucleate giant cells (Fig. 2) suggestive of a herpes group virus infection. *H. hominis* (Type I) was cultured on human kidney tissue culture from the scrotal lesions. A further Tzanck smear taken 2 days later no longer showed the cytology of a virus infection.

He was treated with bland local applications; 3 days later the lesions began to heal. Within a week all that remained were hyperpigmentation and very slight maceration of the involved areas. No recurrences have occurred in the 4 months since this attack.

DISCUSSION

The basic defect in CBFP is a structural weakness of the epidermal cells which is inherited as an autosomal dominant characteristic. The anatomical location of the lesions suggests that repeated minor shearing stress plays a part in producing blisters. This can be demonstrated experimentally by performing a biopsy on an area which has previously been rubbed (Haber & Russell, 1950; Forman & Calnan, 1952). Injury of the other kinds such as freezing, mild burning, chemical damage and allergic contact dermatitis can also reproduce the changes (Chorzelski, 1962). However, the commonest cause of outbreaks, judging by the natural history of the disease, its response to treatment and the results of investigations, is infection with *Staph. pyogenes* (Lowenthal, 1959). Lowenthal points out that acantholytic cells are not specific for pemphigus and CBFP but are also found in impetigo and virus infections. Hailey & Hailey, in their orginal description, described the clinical difficulties in differentiation from impetigo contagiosa.

The recognition of a specific type of *Staph. pyogenes* (phage group II, type 71) as the cause of impetigo contagiosum and of toxic epidermal necrolysis has stimulated several workers to investigate the factors which enable this particular type to produce subcorneal cleavage and epidermal necrolysis. Arbuthnott *et al.* (1972) conclude that 'it seems likely that factors specific to the host rather than the causative organism determine the type of lesion produced'. It is possible that this is true for CBFP also.

Herpes simplex infection is very common in the general population. Antibodies as evidence of past infection are present in 28–96% of people varying with their age and socio-economic background (Juel-Jensen, 1969). An interesting feature of recurrent herpes infection is that it occurs in the presence of circulating antibodies (Hoggan & Roizman, 1959). Formation of giant cells by engulfment or merging of non-infected with infected cells presumably helps the spread of the virus. It is known that the virus is secreted from the pharynx of patients in the absence of lip lesions (Cesario *et al.*, 1969). Foley *et al.* (1970) described herpes simplex infection of burns. This was often not suspected clinically and was in fact diagnosed in their first patient by electronmicroscopy of a thin section from the edge of a lesion, typical herpes virus particles and intranuclear inclusions being seen. Neither virus particles nor intranuclear inclusions have been described in CBFP.

Electron microscopy of the lesions of CBFP suggest that the first change is separation of the tonofilaments from the desmosomes, followed by weakening of the intercellular attachment and by dyskeratosis within the cells (Wilgram, Caulfield & Lever, 1968). It would seem that this change can be precipitated by a variety of stimuli in patients with a genetic predisposition.

Many different types of treatment have been used in CBFP with variable degrees of success. Antibiotics either locally or systemically, or local antiseptics, are not always successful, which suggests that infection with staphylococci or candida is not important in all patients. Several authors (Palmer & Perry, 1962; Ayres & Anderson, 1939) feel that the role of bacteria and yeasts in such lesions is that of a secondary invader rather than the precipitating cause. Sarkany (1959) suggested treatment with Grenz rays (long-wave X-rays) and reported remissions of 4–14 months in six patients. Knight (1972) suggested a similar régime for the prevention of recurrent herpes simplex infections of the skin.

It is difficult to decide whether the herpes virus was the precipitating factor in this man's exacerbation or merely a secondary invader in a damaged area of skin. The early symptoms of a burning sensation and unusually severe pain would favour the former view. Similarly the 10 day duration of symptoms corresponds to the normal duration of herpes simplex attacks (Blank & Rake, 1955) and this suggests early rather than late involvement by the virus.

Herpes simplex infection in our patient was not suspected until the Tzanck smear was examined, and the virus-induced changes would not have been seen if the only smear taken had been the second one. This shows the value of cytological examination and the necessity for performing it early in the outbreak.

It is tempting to combine the reports of beneficial results of Grenz ray therapy in recurrent herpes simplex attacks and in CBFP to suggest that the improvement in CBFP may be due to prevention of precipitation by recurrent herpes simplex. If this is so, early local treatment with idoxuridine may also be helpful.

ACKNOWLEDGMENTS

We would like to thank Dr W. Highman for her help in looking at the Tzanck smears.

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History of Dermatology

A note on Robert Willan's Edinburgh days

P.J.HARE

Department of Dermatology, The Royal Infirmary, Edinburgh

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Robert Willan was born in the North Riding of Yorkshire in 1757. The family home was in a house built by his grandfather in 1713 and recently extensively renovated by the present owner. A barn built by Willan's parents in 1748 suffered damage by an earth tremor in 1970, a reminder that our heritage of historic buildings is not to be taken for granted.

Robert Willan came to Edinburgh in the autumn of 1777 shortly before his twentieth birthday. After the customary 3 years' study he graduated MD in 1780 with a thesis '*On Inflammation of the Liver*'. These are the sole recorded facts about Willan's sojourn in Edinburgh, contrasting with the relative wealth of information about his subsequent career in London.

Why did Willan, an Englishman, come to Edinburgh? There are three obvious reasons:

First, his father graduated here in 1745, his thesis being dedicated to John Fothergill.

Second, the Willans, like the Fothergills who befriended them, were Quakers and would have been excluded from Oxford and Cambridge under the Test Act.

Third, Edinburgh offered unrivalled opportunities for medical education. The great London medical schools were yet to arise, whereas Edinburgh provided a combination of academic and practical tuition not otherwise available in Britain.

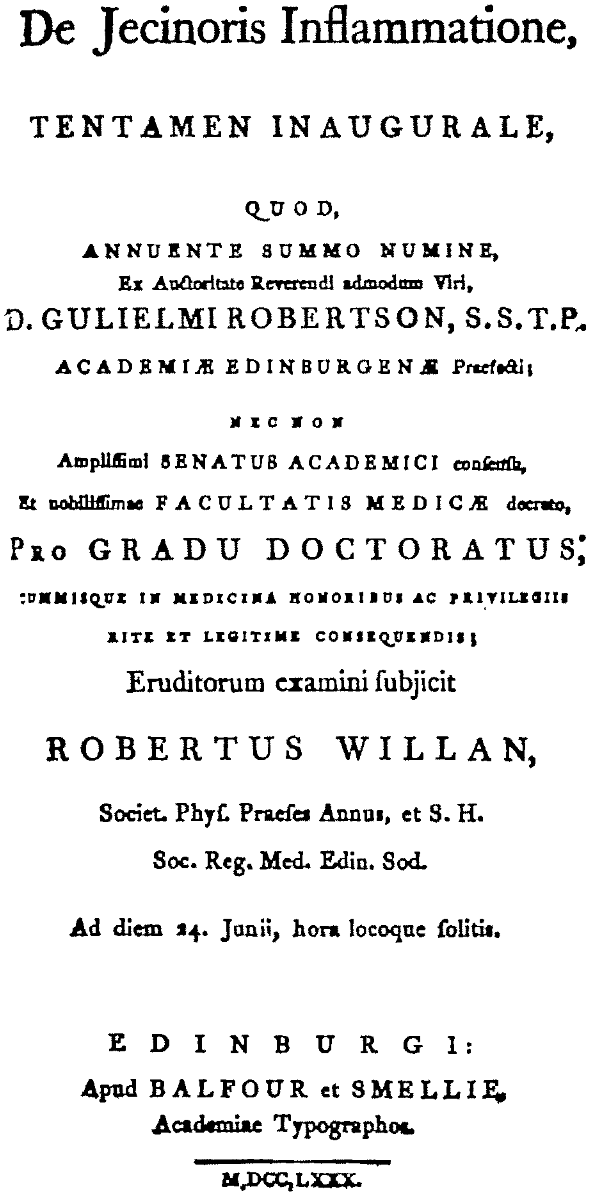
The Medical Faculty of Edinburgh University was founded in the early eighteenth century, and was modelled on that of Leiden, inspired by the example of Hermann Boerhaave. Its fame eclipsed that of its parent and Boerhaave is alleged to have said that he had prayed God would send the Edinburgh professors larger salaries. This was a reference to the fact that their success was attributed to their small stipends, which obliged them to exert themselves to attract and retain students—whom they charged two to three guineas for a course of instruction.

Although at this time the University was housed in old and inadequate buildings, it had a good library. Also the new Royal Infirmary, built in 1739 to the design of William Adam, provided beds for over 200 patients, and medical teachers had privileges within the Infirmary.

Which teachers would have influenced young Robert Willan? He was taught anatomy by Alexander Monro Secundus (three generations of Monros held the Chair of Anatomy for over a century). This Alexander Monro was not only a distinguished anatomist but an excellent teacher, now remembered principally because of the foramen which bears his name. Willan was a brilliant classical scholar, from which we can judge that he had a precise and orderly mind. Monro's teaching would have inculcated the habit of detailed physical observation and analysis.

Botany was then an important medical subject through materia medica. John Hope was Professor of Botany and Superintendent of the Physic Garden. He had interrupted his own medical studies to go to Paris to work under Bernard de Jussieu, a leading exponent of the Linnaean system of classification, which Hope in turn introduced to Scotland. Hope's *Termini Botanici* contains many words familiar to dermatologists.

William Cullen, Professor of the Practice of Physic, was a celebrated teacher and writer of text books for medical students. He applied the Linnaean system to the classification of diseases, as indeed Linnaeus, himself a physician, had already done. Cullen arranged diseases, by symptoms, into Classes, Orders, Genera, Species and Varieties. There is little doubt that the example of Hope and Cullen must have influenced Willan's ideas on the classification of cutaneous lesions.



De Jecinoris Inflammatione,

TENTAMEN INAUGURALE,

QUOD,
ANNUENTE SUMMO NUMINE,
Ex Auctoritate Reverendi admodum Viri,
D. GULIELMI ROBERTSON, S.S.T.P.
ACADEMIÆ EDINBURGENÆ Praefecti;

NEC NON
Ampliſſimi SENATUS ACADEMICI conſenſu,
Et nobiliſſimae FACULTATIS MEDICÆ decreto,
PRO GRADU DOCTORATUS;
SUMMISQUE IN MEDICINA HONORIBUS AC PRIVILEGIIS
RITE ET LEGITIME CONSEQUENDIS;
Eruditorum examini ſubjicit

ROBERTUS WILLAN,

Societ. Phyſ. Praeſes Annus, et S. H.
Soc. Reg. Med. Edin. Sod.

Ad diem 24. Junii, hora locoque ſolitis.

EDINBURGI:
Apud BALFOUR et SMELLIE,
Academiae Typographos.

M,DCC,LXXX.

FIGURE 1. Title page of Willan's thesis for the degree of MD. Edinburgh, 1780.

A fourth influence may have been that of Dr Andrew Duncan, Senior, but here we are in the realms of speculation. Duncan was a true philanthropist; he really loved mankind. He befriended numerous students, entertaining them to tea and talk at a time when staff–student relationships were usually distant. Temporarily prevented from occupying a chair in medicine, he founded a public dispensary for patients who did not require admission to the Infirmary. Here he gave extramural classes for 15 years and was assisted by medical students, some of whose names are recorded. He published '*Medical Cases selected from the Records of the Public Dispensary*' in 1777 with a second edition in 1796. This book includes descriptions of four dermatological cases and his remarks about one of these are interesting. Of this case he said 'there is considerable difficulty in affixing a proper name to it. And indeed, this is an observation which applies to cutaneous affections in general. . . . I cannot help thinking, that cutaneous diseases are not hitherto distinguished with sufficient accuracy, either by nosological or practical writers. . . . It would, I apprehend, be of great consequence in practice, could distinct genera of these be formed, especially if they could be distinguished according to the causes from which they proceed. . . . If therefore, proper marks could be discovered for distinguishing these from each other, I should consider it as a matter of importance. But such marks, I apprehend, have not hitherto been pointed out; and we can only form uncertain conjectures from the appearances and other circumstances'.

I need scarcely remind you that within 5 years of graduating, Willan had read his paper to the Medical Society of London for which he was later awarded the Fothergillian medal, and which was the basis of his principal contribution to dermatological evolution, namely his classification. Also let us recall that soon after his settling in London he was appointed to the Public Dispensary in Carey Street.

These then are the likely influences on Willan's development in Edinburgh. His thesis, written and defended in Latin, is relatively brief, but concise and closely argued. Its title page (Fig. 1) reveals that he was a member and officer of two societies. One was the Royal Medical Society, the oldest existing medical student society in Britain. Not all medical students were graduating students and membership of the Society was then by election from the ranks of the most distinguished. Such members were required to read a dissertation and my enquiry revealed that the records of the Society had been preserved, amongst them a dissertation (1779 ?) by Robert Willan entitled '*Is Mercury a Specific in the Smallpox ?*'. This is evidence of Willan's early interest in the subject of smallpox, an interest he was to retain all his life. He was later to join those in London urging for the creation of a Fever Hospital. He wrote a book '*On Vaccine Inoculation*' in 1806, dedicated to the Chancellor of the Exchequer, recommending the public provision of facilities for vaccination. This book contains a letter from Edward Jenner and reports from various centres practising vaccination, including what had come to be called the Royal Public Dispensary and Vaccine Institute in Edinburgh.

The Physical Society, now the Royal Physical Society, began as another of the numerous professional societies which abounded in Edinburgh in the Golden Era. By repeating the formula used in the case of the Royal Medical Society, although with greater difficulty, it was possible for me to trace two further manuscript dissertations written by Robert Willan in 1779.

Both are in Latin; one is on the human eye and substantiates the theory that Alexander Monro was influential in Willan's intellectual development, and the second, on venesection, reveals a healthy scepticism about treatment.

I hope I have said enough to show that Willan's student days in Edinburgh formed a significant part of his career and that we may be justly proud of the contribution of our medical school to the education of this illustrious man.

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Comment

DISORDERS OF CAPILLARY PERMEABILITY

True capillary permeability, in the sense of unit rate of transfer across a unit area of capillary membrane of unit thickness with a unit pressure gradient across it, is not measurable in man. What can be measured, though, is the overall rate of transfer of substances from the intravascular to the extravascular space, and in practice, of course, it is changes in this bulk transfer which are important clinically, regardless of whether it is the capillary area or thickness or the pressure acting across the membrane which is abnormal. In this review we shall be concerned therefore with overall transfer and not with true permeability, although for convenience we shall use the term capillary permeability. Furthermore, since serum albumin is of special interest in that increased permeability to it is an important factor in the pathogenesis of some oedemas, we shall consider the transfer of this protein only. Serum albumin is found mainly, but not entirely within the intravascular space. A certain amount leaks out through the walls of the capillaries, but protein thus 'lost' is returned, mainly by lymphatic resorption and, under normal conditions, a state of equilibrium exists between the overall 'loss' and the overall return. Any upset in this equilibrium which results in a net increase of albumin in the extravascular space will, if gross enough, result in oedema, and although this upset will not necessarily be an increase in capillary permeability, this is one possibility which can be investigated relatively simply.

Clinical methods of measurement of capillary permeability

(1) *Landis' method* (Landis *et al.*, 1932). This is chiefly of historical interest, but is still in use and has certain advantages over other methods. Of these, the greatest is that it can be used to measure capillary permeability in a known area such as the forearm. The Landis method is based on the assumption that changes in the haematocrit and in the concentration of albumin in the serum during a period of venous occlusion are a measure of capillary permeability to water and albumin respectively, and, roughly speaking, this is so. By the very nature of the method though, the pressures acting on the capillary wall may distort it, and the overall permeability may be influenced accordingly. The Landis method can be made less dependent on changes in the area of the functioning capillary bed by using vasodilator drugs. Jayson & Barks (1971) recently used the method, in a modified form, to demonstrate an increased capillary permeability in rheumatoid arthritis.

(2) *Dilution and plasma clearance methods.* In these methods for measuring capillary permeability, serum albumin is marked with dyes or isotopes, whose disappearance from the plasma is then measured. It is assumed that this rate of disappearance is proportional to the capillary permeability to albumin. This can only be true in the early phase after the injection before there is significant resorption of extravascular isotope, and so such methods involve taking multiple specimens of blood in the first 20 min or so after the intravenous injection. Plasma clearance methods have been used to study capillary permeability in certain disease conditions, and both ^{131}I (Ismail, Khalifa & Madwar, 1965) and T-1824 (Clarkson *et al.*, 1960) have been used as markers.

One consequence of increased capillary permeability is that it invalidates any measurements of the patient's plasma volume which are based on the dilution of labelled protein: but this fact can be turned to good effect, and over-estimated plasma volume can itself be used as an index of capillary permeability. All that is required is that this 'plasma volume' measurement should be compared with the true plasma volume calculated from the plasma dilution of a particle too large to leak out of the capillaries: both large molecular weight dextran (Verel, 1958) and ^{51}Cr-tagged erythrocytes (Marks & Shuster, 1966) have been used for this purpose. These methods have the advantage for the patient that they are simple to carry out, requiring fewer blood samples, but they do introduce a new source of error, especially when erythrocytes are used. Then it has to be assumed that there is no alteration in red cell/plasma distribution within the vascular tree, since the venous haematocrit is used to calculate the red cell dilution.

In the collagen vascular diseases the small blood vessels may be abnormal, as may distribution of red cells and plasma within them, and so in these cases measurement of capillary permeability by a method involving erythrocyte dilution is particularly likely to have its limitations (Marks, Birkett & Shuster, 1972).

One of the greatest disadvantages of all these plasma clearance and dilution methods is that they are 'whole body' methods, that is they measure the sum of many different capillary permeabilities in different parts of the body. Thus a normal result does not exclude a local increase in permeability and an abnormal result gives no indication of the site of the 'leak'.

Clinical syndromes in which capillary permeability is increased

The oedema in the vicinity of the lesions of urticaria and various other inflammatory skin conditions is well known to be associated with a localized increase in capillary permeability, but only more recently has much attention been paid to more generalized oedema occurring often at a distance away from the lesions in some of these diseases and to the part played by increased capillary permeability in spontaneous periodic oedema.

Oedema in eczema and psoriasis

This is most common and most severe in patients with erythrodermic eczema or psoriasis, but it occurs in patients with less extensive rashes too. Capillary permeability is found to be increased in these conditions, and this increase is roughly proportional to the extent of the rash (Marks & Shuster, 1966). The oedema varies in severity from a little pitting oedema of the ankles to gross oedema of the whole body. Occasionally in erythroderma this oedema may be of very sudden onset and rapid progression, and it may be accompanied by collapse from hypovolaemia and hypotension. Patients with this syndrome constitute a dermatological emergency, and death may occur from the increased capillary permeability to protein (Marks & Shuster, 1966; Zak and Pai, 1968). Once the condition is diagnosed it should be treated with intravenous infusions of hydrocortisone and concentrated serum albumin (Shuster & Marks, 1970).

Oedema in collagen vascular diseases

Patients with collagen vascular diseases, including disseminated lupus erythematosus, systemic sclerosis, dermatomyositis and rheumatoid arthritis, sometimes have an unexplained oedema. It seems likely that this is due to an increased permeability to serum albumin which many of these patients show (Jayson & Barks, 1971; Marks *et al.*, 1972). The amount of oedema varies from patient to patient: sometimes it is episodic and sometimes it is the presenting feature antedating all other signs and symptoms of the disease (Marks *et al.*, 1972). Some patients with collagen vascular diseases have an increased capillary permeability to serum albumin, even though they do not have oedema. In most of these patients the abnormality is of a lesser degree than in those who do have oedema (Jayson & Barks, 1971; Marks *et al.*, 1972), and presumably the resorption of protein and fluid from the tissue compartment back into the circulation can keep pace with the leakage, so that no disequilibrium exists.

Spontaneous periodic oedema

Attacks of generalized oedema may occur in people who are apparently healthy in other respects. In females they may occur pre-menstrually, but they may also occur quite independently of the menstrual cycle (Clarkson *et al.*, 1960), and they may occur in men (Weinbren, 1963). An increased capillary permeability to protein has been demonstrated in some of these patients (Emerson & Armstrong 1955, Clarkson *et al.*, 1960; Marks & Shuster, 1966; Lagrue, 1972). Although a patient has been reported to have died of her disease (Clarkson *et al.*, 1960) the outcome is usually less disastrous. Treatment is, however, unrewarding and, in particular, response to diuretics is unsatisfactory. Associated with the oedema there may be hyperaldosteronism (Weinbren, 1963), postural hypotension (Streeton & Speller, 1966) and an increased catabolism of albumin (Gill, Waldmann & Bartter, 1972). None of these various abnormalities is likely to be the primary one, and a primary increase in leakage of plasma protein from the intravascular compartment, such as would result from increased capillary permeability, fits the known facts best. The frequency with which increased capillary permeability can be demonstrated in the collagen vascular diseases, and the finding that patients with disseminated lupus erythematosus and systemic sclerosis may actually present with 'spontaneous' periodic oedema at a time when there is no other evidence of their underlying disease (Marks *et al.*, 1972) suggests that it is worth while investigating all cases for evidence of a collagen vascular disease. It may be that 'spontaneous' periodic oedema is an early stage of one of this group of diseases.

JANET MARKS
SAM SHUSTER

Department of Dermatology,
University of Newcastle-upon-Tyne

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Obituaries

FREDERICK C. FLORANCE

The many friends of Fred Florance were sorry to hear of his death in his eightieth year after a long illness. He was elected a member of our Association (N.S.W. Branch) in 1935 and greatly appreciated being chosen as an Honorary Member.

He was late in starting his career as a dermatologist, for he was only fourteen when his father died and he had to earn his living as a journeyman printer. In 1916–18 he served in France as a staff sergeant before being invalided home badly gassed. On his recovery he became a medical student and graduated in Sydney in 1924. After post-graduate work, he determined to study dermatology and in 1929 was appointed honorary assistant dermatologist to the Royal North Shore Hospital in Sydney. He came to this country and to Denmark for post-graduate work, returning home in 1934. Much of his time in Britain was spent in Edinburgh, where we soon appreciated his sterling character and delightful dry sense of humour. For example, when an ingenuous youth said that he supposed that there were a lot of Scotsmen in Australia, Fred replied that he reckoned that really rabbits were the biggest pest.

He became honorary dermatologist and head of the Skin Department of the North Shore Hospital, and one of the outstanding figures in Australian dermatology. A foundation member of the Dermatological Association of Australia and of the Australasian College of Dermatologists which replaced this, Dr Florance was a wise, kindly mentor and colleague, full of common sense and deep practical knowledge. He loved to visit this country to attend our Annual Meetings, to renew old friendships and form new ones. He was blessed in a happy family life, and we sympathize with his widow and his sons Dick and Brian. It was a great pleasure to him that Brian followed so well in his dermatological footsteps.

G.A. GRANT PETERKIN

EDWIN BAYLIS ASH

Dr Baylis Ash, Honorary Consultant Dermatologist to the United Birmingham Hospitals, died on 9 November 1972 at the age of 79.

Edwin Baylis Ash was born on 3 September 1893 in Birmingham. His father and mother were both members of old Midland families—and, indeed, in later years he was able to trace a line of descent from Dr John Ash, the eighteenth-century founder of the Birmingham General Hospital. His early education was at King Edward's School, Birmingham. It is likely that the family business—surgical instrument and artificial limb making—created an interest in medicine, and on leaving school he became an undergraduate of the faculty at the University of Birmingham. In 1917, immediately after graduation with the Conjoint diploma, he joined the RAMC, serving principally on the Italian front. After demobilizaton in 1919, with the rank of captain, he held residential appointments as house surgeon at both the General and the former Queen's Hospital, Birmingham, and in addition gave anaesthetics at the Dental Hospital. In 1923 he became M.R.C.P., London. Then followed a period on the staff of the Wilhelminenspitals, Vienna. In 1926 he was awarded a doctorate of medicine of the University of Vienna for a thesis on gastric ulceration and syphilis. On his return to England he was appointed consultant dermatologist to the Skin Hospital, Birmingham, the West Bromwich and District General Hospital, and later the United Birmingham Hospitals. In addition, he acted as pathologist to the Skin Hospital, clinical lecturer in dermatology to the University of Birmingham, member of the Typhus Panel, and, from 1948, member of the Medical Appeals Tribunal of the Midland Region. He retired from the active consultant staff of the United Birmingham Hospitals in 1958 and from the Skin Hospital 3 years later.

Baylis Ash was a small rosy-cheeked man, displaying a puckish sense of humour and an incessant activity. His outpatient work at the Skin Hospital was famed for the prodigious number of patients attending, figures in excess of 300 during an afternoon clinic being recorded from time to time. No call for his services was ever declined, and it is remembered that in the midst of one such clinic a message from an adjacent cinema resulted in his temporary absence for the purpose of supervising a childbirth! Ward rounds were daily affairs and frequently commenced at 8 a.m. On Sunday mornings after the round he often visited the homes of his older patients. His handwriting was legendary for its indecipherability: it has been related that a certain prescription produced a tonic on the first occasion and a liniment on the second! Although essentially a clinician, the histological service of the Skin Hospital was an additional interest which he pursued for many years. Research did not figure prominently in his career, yet he found time to read a paper on the bacteriology of impetigo at the 11th International Congress of Dermatology in 1957. Baylis Ash left himself very little time for hobbies, but when he could be spared from his professional commitments, colour photography and watch collecting were his main interests.

The later years were borne with an uncomplaining courage. He nursed his wife devotedly through a very long and ultimately fatal illness. Later, Parkinson's disease and retinal detachment, resulting in almost total blindness, were further burdens which he bore with a stoical fortitude. He spent the last 2 years of his life in a private home for professional people, where he did his best to contribute to the social welfare of his companions. Although the majority of his colleagues have pre-deceased him, there are still many patients in the Midlands who speak with affection of 'Dr Bayley Ash', as they knew him. He is survived by his son and daughter.

E.A.FAIRBURN

Book Reviews

Review on Hypersensitivity to Drugs. Vol. 1. MAX SAMTER AND C.W.PARKER (Section Eds.), (1972) Oxford: Pergamon Press. Pp. 439 + Drug Glossary, 77 illustrations. Price £12.00.

This is the first volume of a proposed mammoth tome on pharmacology and therapeutics. The obvious disadvantage of such a project in a dynamic and expanding field is that much of the contents of the early volumes will be outdated by the time the final volumes are completed.

This volume is divided into two main sections, the first, dealing with immunology, is comprehensive but rather difficult to follow at times. The fluency of a single textbook on immunology is missing, but this is to be expected as individual chapters are written by different experts. The difficulties of presenting a concise account of a new and developing subject like immunology are stated, but no real attempt is made to overcome them. A synopsis of current views would have been a useful addition at the end of each chapter.

The chapter by B.N.Halpern, in which he describes methods of detecting antibodies produced by drugs, is well presented and comprehensive.

The second section of the book is designed mainly for the clinician, and deals with drug reactions affecting the major systems of the body. Each system is dealt with in a separate chapter; this has the disadvantage of making it difficult to discover reactions to individual drugs without switching from one chapter to another, although a terminal glossary on blue paper makes this task somewhat easier.

Haematological, cutaneous, renal and hepatic systems are thus dealt with comprehensively in separate chapters. One presumes that the cardiovascular, gastrointestinal, and nervous systems will be covered in other volumes. The chapter on drug-induced skin reactions is by R. Schuppli. He mentions the difficulties of proving that a particular skin reaction is due to one drug rather than another, or to the disease for which the drug was given. All the recognized drug-induced skin reactions are well described, with the notable exception of lichenoid rashes.

The final chapter by C.W.Parker is concerned with the practical aspects of diagnosis and treatment of patients who are hypertensive to drugs.

References are many and well selected. Some 1500 authors are listed in the author index.

When completed this work will form an encyclopedia of pharmacology and therapeutics which will be essential as a reference for every library. It will probably be too repetitive for individual purchase.

JOHN ALMEYDA

The Year Book of Dermatology 1972. F.D.Malkinson and R.W.Pearson (Eds.) Chicago: Year Book Medical Publishers. Pp. 416, 67 figures. Distributed in Europe by Lloyd-Luke (Medical Books) Ltd, London. Price £7.00.

The latest year book reviews the literature up to December 1971. The format and arrangement are those introduced by the present editors last year. A vast and impressively international range of journals has been covered and the resulting collection of critical abstracts is better than ever, and is indispensable to the active dermatologist however good his library facilities. The subject of the review article is epidermal cell kinetics, on which Dr Malkinson writes with authority and clarity.

Arthur Rook

Histopathologie der Haut. H.Gartmann and H.Tritsch (1972) Berlin and New York: W. de Gruyter. Pp. 54, 57 illustrations. Price £5.40.

This second issue completes the dermatological section of Kaufmann and Staemmler's *Lehrbuch der Speziellen Pathologischen Anatomie*, produced as an offprint including an index. Most of it is devoted to disorders grouped as keratodermas and rather over-elaborately classified. Here one finds not only the ichthyoses, follicular hyperkeratoses and palmo-plantar syndromes, but Darier's disease, epidermodysplasia verruciformis, knuckle pads and chondrodermatitis helicis. As before, the quality of the black and white photomicrographs is outstandingly good: the text is terse and documentation adequate.

P.J. Hare

Scabies. Kenneth Mellanby (1972) Hampton: Classey. 2nd Edition. Pp. 81, 10 illustrations. Price £1.25.

Dr Mellanby's classic monograph on scabies was first issued in 1943 as one of a series of *Oxford War Manuals*. It contained a critical review of the parasitology, epidemiology, clinical features and treatment of scabies, based to a large extent on the author's own investigations on volunteers. The increase in the incidence of scabies during the last 10 years has prompted Dr Mellanby to reissue the book. This second edition is essentially a reprint of the first, with a new preface in which the author claims that because of the relative rarity of scabies until recently, little has been added to our knowledge of the disease in 30 years. This is to some extent true, but it ignores the valuable contributions of Heilesen, whose monograph was published in Denmark in 1946, and of Borda, whose meticulously conducted investigations were reported in Buenos Aires in 1955. There have also been many minor, but far from insignificant, additions to our knowledge of this infestation. Most dermatologists would surely have preferred to see the second edition of Mellanby's monograph brought fully up to date, but will nevertheless be grateful for this reprint.

Arthur Rook

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Quarterly Review

THERAPEUTICS XXII

Drug Interaction. I

Drug interaction presents an increasing hazard in modern therapy. It is becoming apparent that a large number of drugs interact. The sites of interaction may be at any point during administration, absorption, distribution and eventual elimination of the drug by metabolism or excretion. Interactions between drugs occurring *in vivo* may result in a planned beneficial effect, but not infrequently such interaction is responsible for an adverse effect which may be life-threatening or lethal. The more drugs a patient is taking, the greater is the risk. Patients often take preparations which are available without prescription, such as analgesics, antacids, purgatives and cough medicines. These preparations contain compounds which may interact with prescribed drugs and cause a potentially serious adverse reaction. Pharmacologically active compounds are present also in certain foods and may interact with prescribed drugs, e.g. tyramine in cheese with the monoamine oxidase inhibitor drugs.

Ignorance and apathy as to the hazards of powerful modern drugs may well result in iatrogenic disorders and therapeutic disasters. It is essential for all who prescribe drugs to have an adequate knowledge of their pharmacology and to be aware of potentially harmful interactions. The prediction of all possible drug interactions may not be feasible even with the most thorough animal studies. Often it is only after a drug has been in use for a number of years that its interactions become recognized and the associated risks evaluated. The reporting of adverse drug reactions to the Committee on Safety of Drugs is of considerable help in this respect.

It is hoped that this review will serve as a stimulus for further reading on this problem (Martin, 1971; Dollery, 1972; Herxheimer, 1969).

SITES OF DRUG INTERACTIONS

These can be classified according to where the interactions occur.

(1) Outside the body, e.g. in the infusion bottle.
(2) In the gut.
(3) In the plasma.
(4) At the receptor site.
(5) On binding to tissue proteins.
(6) During metabolism in the liver.
(7) During excretion by the kidney.

Alternatively, drug interactions can be regarded as of two kinds: firstly where interactions alter the response to a drug without affecting either its concentration in the plasma or at the receptor site; secondly, where interactions result in an alteration in the concentration of the drug so that the effect is either increased or diminished. While the first kind of interaction is often predictable, the latter usually not. In addition, interaction can be expected when drugs of similar action are prescribed.

(1) *Outside the body*

Interactions between drugs may occur when compounds are added to solutions for injection and infusion. Additions to infusions may alter their physical state, for example fat emulsions are broken down by electrolytes. Suxamethonium chloride reacts chemically with thiopentone and pentobarbitone. A further hazard is the mixing in the same syringe of soluble with protamine zinc insulin.

(2) *In the gut*

The absorption of drugs may be affected by interactions which alter the function of the gastro-intestinal tract, the physicochemical characteristics of its contents, the mucosa, and the transport mechanisms, both active and passive, concerned with the movement of drugs from the lumen of the gut into the body fluids. Atropine-like drugs, codeine, morphine and other opiates can delay gastric emptying and thereby slow the intestinal absorption of drugs given by mouth. The absorption of some drugs may be affected and markedly diminished by the formation of insoluble complexes. Thus tetracycline forms non-absorbable complexes with such cations as aluminium, calcium or magnesium. Antacids, such as aluminium hydroxide, milk of magnesia and sodium bicarbonate, also diminish absorption of tetracycline. Phytic acid in the food forms complexes with calcium and iron. Alteration of the bacterial flora of the gut by antibiotics may affect a patient's susceptibility to a drug, e.g. reduction of the vitamin K synthesizing flora in the gut by antibiotics may cause disastrous bleeding when patients are on anticoagulants. Drugs such as methyldopa may be slowly absorbed in the gut as a result of competition with natural amino acids for the same transport site and mechanism.

(3) *In the plasma*

Once absorbed, most drugs are distributed first between the plasma and its proteins and then between the extracellular fluid, tissue proteins and body fat. In the intravascular compartment, some of the drug will be ionized and some non-ionized, the proportion depending upon the pH and the electrolyte composition of the plasma. Any one drug which alters these factors may change the degree of ionization of another. Ionization and the lipid solubility of the drug determines its distribution between plasma and body fat, since non-ionized drugs are more easily absorbed by fat. Most biological membranes are lipid bi-layers, and non-ionized drugs can penetrate them more easily than ionized. This is of considerable importance with drugs which pass the blood–brain barrier. Phenobarbitone, a weak acid, will cross the blood brain barrier more easily and cause deepening unconsciousness in acidotic individuals because its ionization is suppressed.

The distribution of drugs to tissues will depend not only upon lipid solubility but also on the molecular size of the drug, the capillary bore and the intercellular permeability. The total molecular size of a drug may be altered by protein binding (cf. section 5). The diameter of capillaries is altered by many drugs, such as adrenaline, nor-adrenaline, oestrogens and histamine, and these can affect the action of administered drugs. Alterations in capillary permeability will affect the movement of drugs from the vessel to the extravascular space.

Some drugs exhibit a specificity in their binding to certain proteins and any drug which competes for these binding sites will clearly alter its action. Drugs binding more tenaciously to protein displace those less firmly bound.

(4) *At the receptor*

Interaction between drugs can occur at the same receptor site and the action of the drug may be enhanced or inhibited. A complex but common interaction is the potentiation of the sedative action of antihistamines and barbiturates by alcohol. Potentiation of exogenous noradrenaline occurs when a patient is on such drugs as guanethidine and bethanidine, since these drugs deplete adrenergic nerves of noradrenaline and thus make the receptor sites super-sensitive. The blockage of receptors is an interaction mechanism frequently used in therapy: the action of noradrenaline is inhibited by such drugs as phenoxybenzamine, and β-adrenergic blockers such as propanolol compete with endogenous adrenaline and noradrenaline at myocardial β-adrenergic receptor sites. Competitive antagonism between folic acid and methotrexate, and morphine and nalorphine are further examples. Streptomycin and neomycin have a curare-like action and these drugs potentiate tubocurarine and gallamine.

(5) *Protein binding*

Many drugs are reversibly bound to plasma proteins, notably albumin, though lipid soluble molecules such as vitamin D are largely bound to lipoproteins.

The fraction of the drug bound to protein is inactive and largely unavailable for metabolism or excretion by the kidney. This implies that protein bound drugs have a prolonged activity within the body. The protein bound fraction is in equilibrium with the active unbound drug and the relative concentrations are dependent on the administered dose of the drug. Thus it is possible to saturate all protein binding sites available for a particular drug and this may result in a rapid rise in the concentration of free drug. Displacement of one drug from its protein binding site by another is an extremely important drug interaction. Only a slight displacement of a bound drug such as bishydroxycoumarin by clofibrate (Atromid-S) or butazolidine will greatly potentiate its anticoagulant effect.

Further, some naturally occurring substances, for example bilirubin, are protein bound and can be displaced by drugs. Infants with neonatal jaundice may develop kernicterus as a result of the administration of sulphonamides or salicylates which displace the protein bound bilirubin, freezing it to enter the tissues.

(6) *In the liver*

Drugs may induce or inhibit microsomal enzymes liver cells.

Enzyme induction. Many hundreds of drugs, including analgesics, antihistamines, anticonvulsants, anti-inflammatory agents, hormones, hypoglycaemics, sedatives and tranquillizers stimulate the metabolism of themselves and other drugs by the induction of microsomal enzymes in the liver. The induction of such enzymes by drugs is becoming recognized as one of the major problems of drug interaction. One drug alone, phenobarbitone, stimulates the metabolism of more than sixty chemicals (Conney, 1967, 1969). A severe haemorrhage may result from discontinuing phenobarbitone when a patient has been well controlled by an oral anticoagulant. Similarly, this may occur when a patient stops taking griseofulvin whilst remaining on oral anticoagulants.

Enzyme inhibition. The list of drugs which inhibit microsomal enzymes is similarly expanding rapidly (Martin, 1971). Inhibition of the oxidation of tolbutamide by sulphonamide, which competes for the same microsomal enzyme, may lead to hypoglycaemic attacks due to an accumulation of tolbutamide in the plasma.

(7) *In the kidney*

Alterations in glomerular filtration, tubular secretion and tubular reabsorption can occur from drug interactions. If the glomerular filtration of a drug is increased, the level in the blood is lowered and its action and duration of effect are diminished. Drugs which diminish glomerular filtration and tubular secretion will lead to elevated blood levels and a more prolonged action. In therapy, probenecid is used to diminish the excretion of penicillin by the kidney, thereby maintaining high blood levels. Probenecid is also used to enhance the excretion of uric acid in the treatment of gout. Salicylates have a uricosuric action when used alone, but will inhibit the uricosuric effect of probenecid and also of phenylbutazone.

Changes in the pH of the urine by the use of alkaliinzing and acidifying agents profoundly affect the excretion rates of a large number of drugs.

Many drugs interact at different sites in the body and involve various mechanisms. In a subsequent review the interactions of a number of drugs commonly used in dermatology will be discussed.

S. S. Bleehen

United Sheffield Hospitals

I. R. Edwards

University Department of Therapeutics,
Sheffield

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News and Notices

Royal College of Physicians of London

The following members of the British Association of Dermatology have recently been elected to the Fellowship of the Royal College of Physicians of London:

R.G. Howell, D.L. Rees, R.H. Seville, J.C. Mitchell, Group Captain J.N.S. Mitchell, R.H. Champion, Harvey Baker.

British Journal of Dermatology

All enquiries regarding subscriptions to the *British Journal of Dermatology* should be sent to the Publishers, Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL.

Investigative Group of the British Association of Dermatology

The next meeting of the Group will be held at the University of Liverpool on Friday and Saturday, 11 and 12 January 1974.

Those requiring accomodation, which on the Friday is available in a Student's Hall of Residence, should write to Dr C. F. H. Vickers, Department of Dermatology, Liverpool Royal Infirmary. Members wishing to submit papers should send standard abstracts (details are being pre-circulated) to Dr M.W. Greaves, Department of Dermatology, Royal Victoria Infirmary, Newcastle-upon-Tyne, not later than 15 October 1973. There will also be facilities for scientific demonstrations, and proposals for these should reach Dr Greaves or Dr Vickers by the same date.

Conference on *Myobacterium ulcerans*

A Conference on *Mycobacterium ulcerans* will be held at the Middlesex Hospital Medical School on 5–6 July 1973. This Conference is supported by the National Foundation for Research into Crippling Diseases. The Conference fee, which also covers the cost of luncheon and coffee for the two days, will be £3.50. Particulars may be obtained from Dr J. L. Stanford, Department of Pathology, Middlesex Hospital.

University of Cambridge Postgraduate Medical School

The fourth of the Courses held at 5 yearly intervals and entitled 'Progress in the Biological Sciences in Relation to Dermatology' will take place in Cambridge from 24 to 29 September 1973. The Course is intended primarily for clinicians of considerable experience, but anyone who is interested is welcome to attend. Details of the programme and accomodation may be obtained from the Secretary of the Medical School, J.W. Woodcock, M.A., Hills Road, Cambridge.

Prizes of the Spanish Academy of Dermatology

Two prizes, each of 15,000 pesetas, will be awarded for original unpublished work, on any topic on dermatology or venereology, submitted by 1 December 1973.

The Sainz de Aja prize is restricted to citizens of Spain and the Latin American countries. The Spanish Academy prize is open to dermatologists of any nationality. Further particulars may be obtained from the Spanish Academy of Dermatology, Calle Sandoval, 7, Madrid.

APPOINTMENTS

Nottingham Area

Dr P.D.C. Kinmont has been appointed Consultant Dermatologist to the Nottingham General, City and

Children's Hospitals. He was educated at Epsom College and at King's College Hospital, London, where he qualified M.R.C.S., L.R.C.P., and M.B., B.S. in 1939. After a house surgeon appointment at this hospital, he served as a Territorial Army Officer in the R.A.M.C. from 1939 to 1945, during which time he was awarded the M.B.E. and was mentioned in despatches.

He returned to King's College Hospital in 1946 as Dermatological Registrar, and in that year proceeded M.D. and M.R.C.P. (London). He was admitted F.R.C.P. (London) in 1965. In 1947 he was appointed Consultant Dermatologist to the Derbyshire Royal Infirmary and Derbyshire Hospital for Sick Children. During his service in Derby, Dr Kinmont has been President of the East Midlands Society of Physicians (1964), President of the North of England Dermatological Society (1969/70), Chairman of the Midlands Dermatological Society (1970) and Vice President of the Section of Dermatology of the Royal Society of Medicine (1971–1973).

He has published numerous papers on clinical dermatology and on cosmetology.

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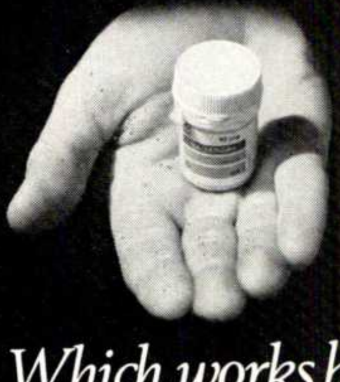

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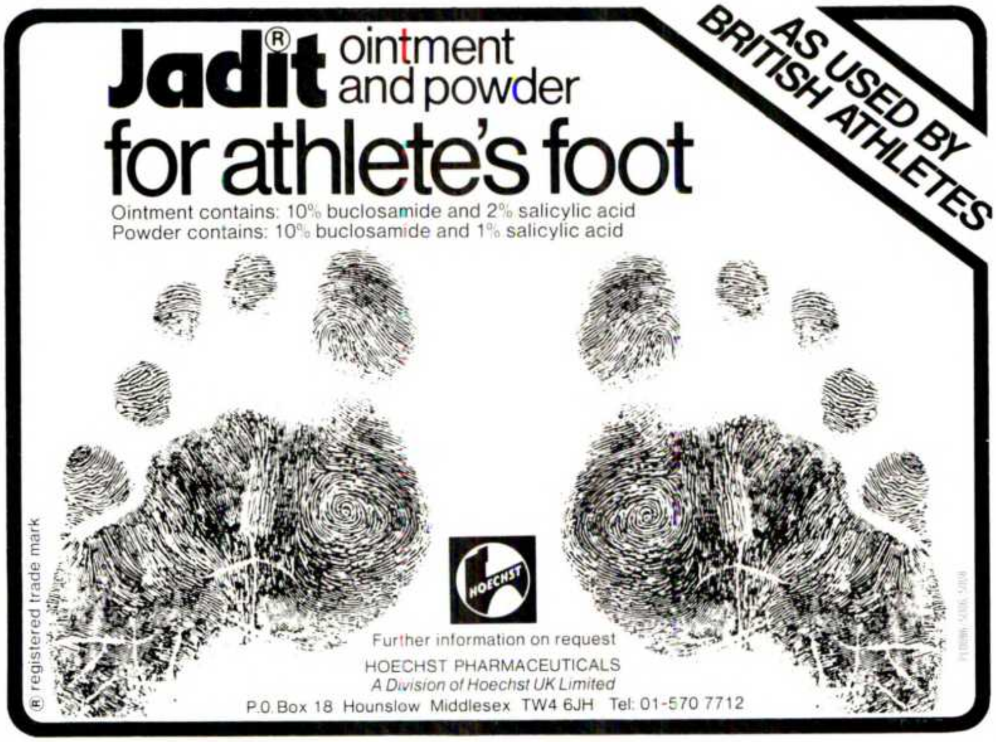
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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	**g**
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	percent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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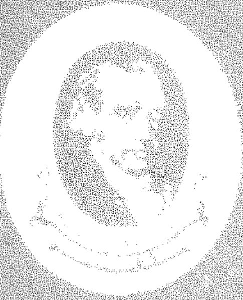

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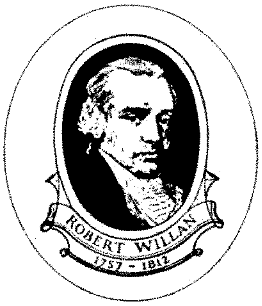

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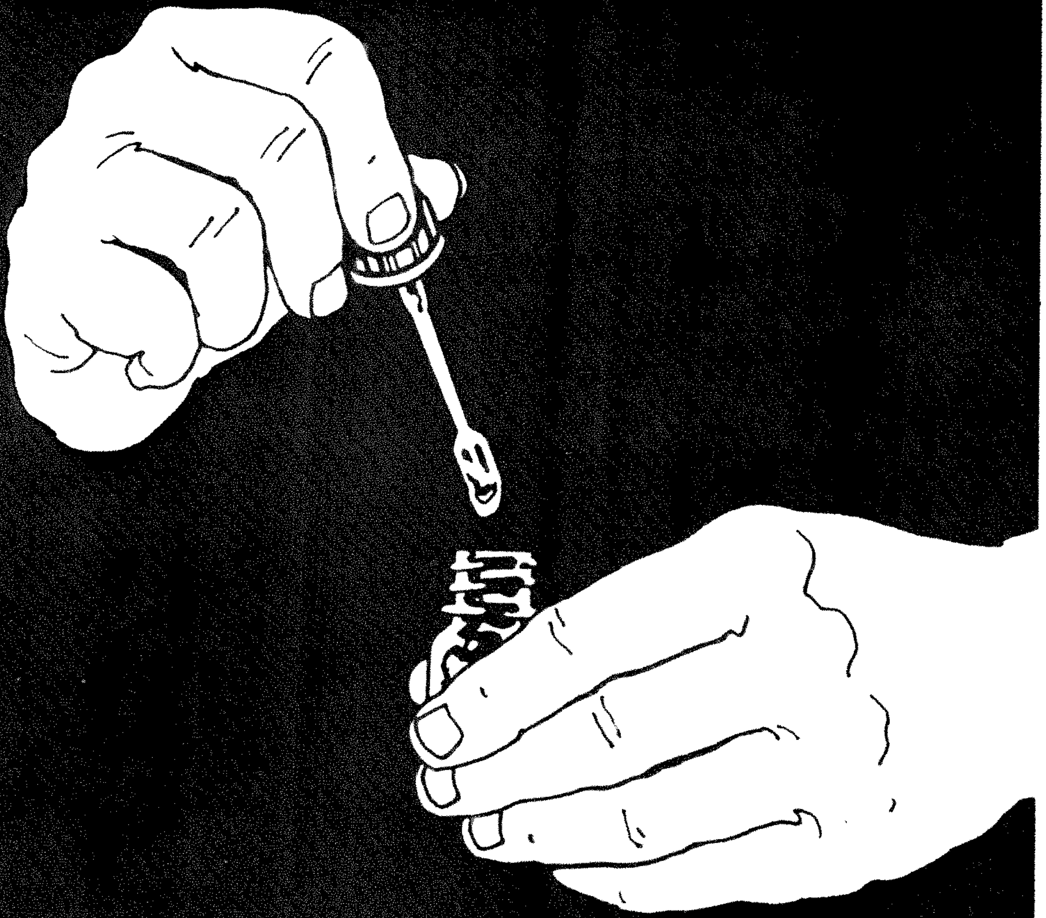

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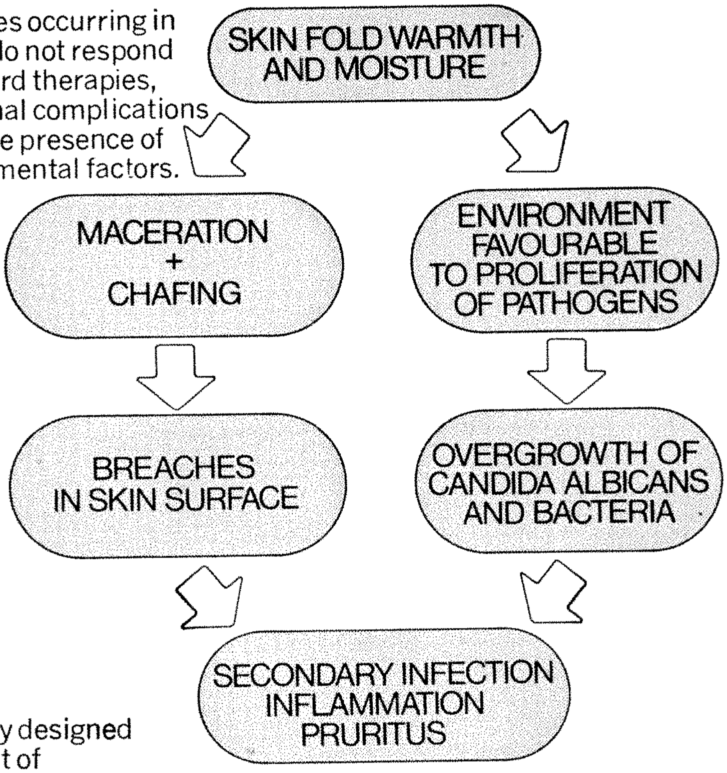

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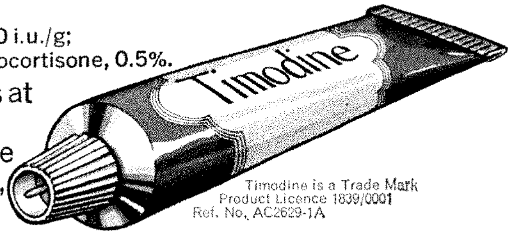

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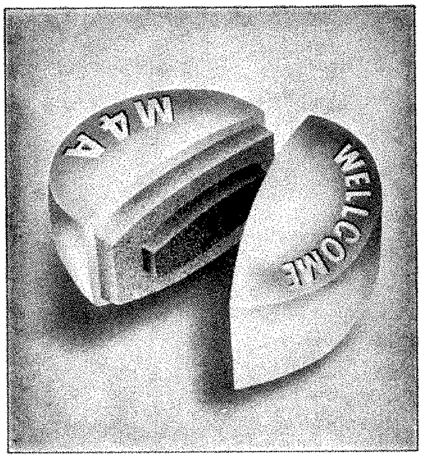

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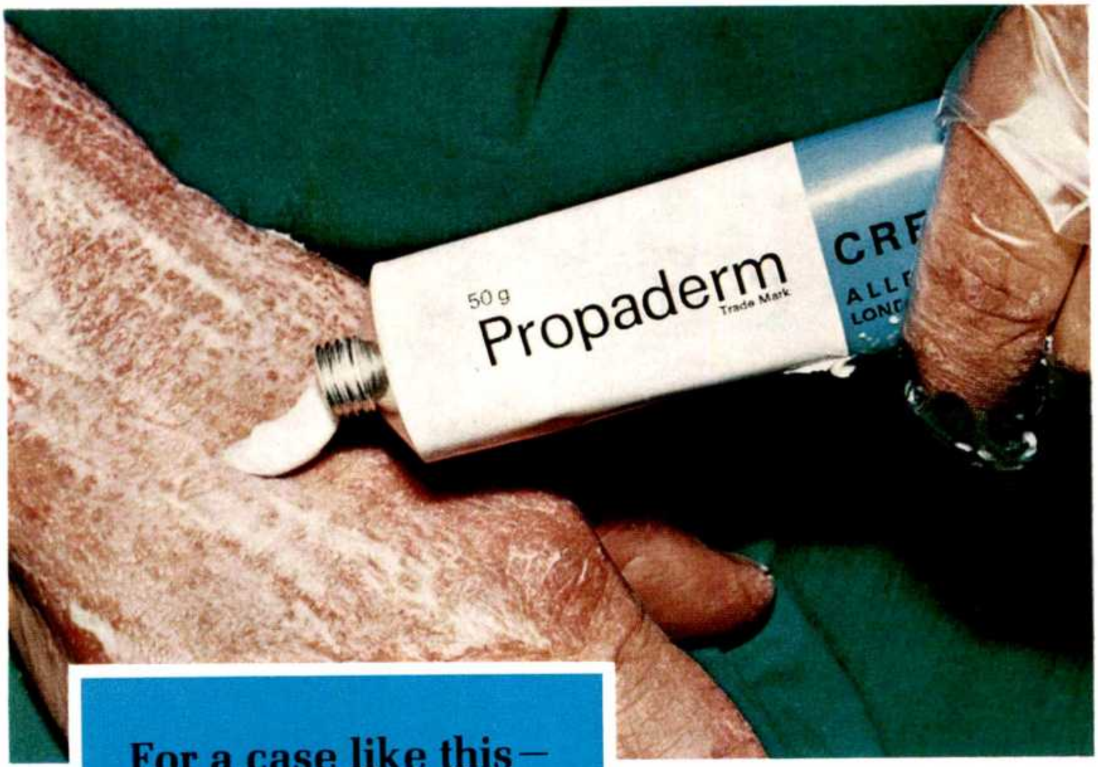

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British Journal of Dermatology (1973) **89**, 1.

Clinical and Laboratory Investigations

Tubular structures in affected and normal skin in chronic discoid and systemic lupus erythematosus: electron microscopic studies

UWE-FRITHJOF HAUSTEIN

Department of Dermatology, University Hospital (Charité), Berlin, G.D.R.

Accepted for publication 13 January 1973

SUMMARY

Biopsy specimens of involved and clinically normal skin of thirty-seven patients suffering from different forms of LE were examined ultrastructurally to determine the presence and distribution of tubular structures. They were localized in endothelial cells, histiocytes, macrophages, fibroblasts, keratinocytes and in lymphocytes of the dermal infiltrates, and were mostly intracellular. A correlation was found between their frequency and the acuity of the lesions, but not with previous treatment of the disease, either with steroids or with chloroquine. The involved skin was affected in all cases of systemic lupus erythematosus and severe subacute and disseminated variants of chronic discoid lupus erythematosus, but in only eleven of seventeen cases of the localized form. In clinically normal skin all specimens of SLE examined showed positive results, there were fewer positives in the subacute and the disseminated forms of CDLE and only one positive in localized CDLE. Hypotheses on the origin, nature and significance of the tubular structures are discussed. They are suspected to be unidentified virus particles rather than a reaction product of a virus infection.

Genetic, immunological and viral factors appear to be involved in the pathogenesis of lupus erythematosus (LE) (Sönnichsen, Haustein & Barthelmes, 1971; Ziff, 1971). The genetic background is evidenced by the increased incidence in families. The immunological factors are demonstrated by depression of the cellular immunity and by a moderate overall elevation of humoral immunity. The latter is manifest by increased antibody titres to certain antigens, by the widespread depostion of immune complexes (Grishman & Churg, 1970), and by a variety of immunological phenomena: e.g. LE-factor, antibodies to various forms of DNA and to double-stranded RNA, circulating anti-coagulants, anti-cardiolipin, rheumatoid factor, red cell antibodies, antibodies against cell membrane constituents (of lymphocytes) and 'autoaggressive' lymphocytes with cytotoxic properties (Lamon & Bennett, 1970; Stastny & Ziff, 1971; Stollar, 1971).

During the past 5 years unusual membrane-bound cytoplasmic inclusions composed of tubules with a hollow core have been repeatedly described by electron microscopists. On morphological grounds these structures have been attributed to the replication of myxo- or paramyxoviruses or their effects, suggesting an important role for them in the pathogenesis of LE similar to the 'slow virus infection' by murine leukaemia viruses in the LE-like autoimmune disease of NZB-mice and their hybrids (Barthelmes *et al.*, 1973; Mellors, 1971). The number and variety of diseases, in which the tubular 'paramyxovirus-like' structures have been observed, is constantly increasing (Uzman, Saito & Kasac, 1971). However, at present their origin, nature, specificity and possible significance are still obscure.

Tubular structures have mostly been found in the involved skin although there are a few reports of inclusions in normal skin (Garancis *et al.*, 1971; Nieland, Hashimoto & Masi, 1972; Prunieras *et al.*, 1971). In the present paper we systematically examined electron microscopically both the involved and the normal skin of thirty-seven patients suffering from chronic discoid or systemic lupus erythematosus (CDLE or SLE) in order to determine the distribution of tubular structures and correlate their presence with the acuity and duration of the dermal lesions and with previous treatment of the disease.

MATERIAL AND METHODS

Material

The classification of the diagnostic subgroups was based on clinical, histological and immunofluorescent and other laboratory criteria: nine patients with SLE (nine biopsies from involved, six from normal skin), twenty-eight patients with CDLE (twenty-eight biopsies from involved, eighteen from normal skin), including three patients with the severe subacute form, eight with disseminated discoid lupus, and seventeen with localized lesions. Furthermore, eight control specimens from healthy subjects were examined.

Methods

Specimens were immediately sliced into 1 mm blocks, fixed in 2·5% glutaraldehyde in cacodylate buffer (0·1 N, pH 7·2) for 30 min, washed with the same buffer three times, postfixed in osmium tetroxide for 1 h (1% in cacodylate buffer: 0·1 N, pH 7·2), dehydrated through graded concentrations of ethanol and acetone, stained with 1% uranylacetate in 50% ethanol for 15–30 min immediately after 50% ethanol dehydration, embedded in Vestopal R, sectioned by the ultratome LKB, stained by Reynolds lead citrate solution and examined in the electron microscopes SEM III (VEB Fernsehelektronik Berlin) and Elmiskop 1a (Siemens) at 60 to 80 kV. The pathological changes were also examined on semi-thin slices stained with toluidine blue or methylene blue and basic fuchsin (Morgenroth, Schröder & Themann, 1970) in order to evaluate the blood vessels of the subpapillary plexus, the necrobiotic regions of the basal layer and of the corium, and the lymphocytic infiltrates of the corium.

RESULTS

Localization of tubular structures

The tubular structures were localized predominantly in the endothelium of arterioles, venules and capillaries of the subpapillary plexus (Fig. 1). They were also found in histiocytes, macrophages (Fig. 2) and fibroblasts (Fig. 3) and occasionally in lymphocytes (Fig. 3) in the dermal infiltrates. These structures were also found in lower keratinocytes and in the basal cells (including necrotic basal cells) (Fig. 4). They were observed partly free within the cytoplasm, occasionally in contact with lysosomes and near mitochondria, mostly within the dilated cisternae of the endoplasmic reticulum or in perinuclear regions. They were partially surrounded by a single, or occasionally by a double, and rarely by a multiple-layered membrane, which appeared more or less continuous, often in connection with the endoplasmic reticulum, but free of ribosomes. In no case did they occur within the nucleus.

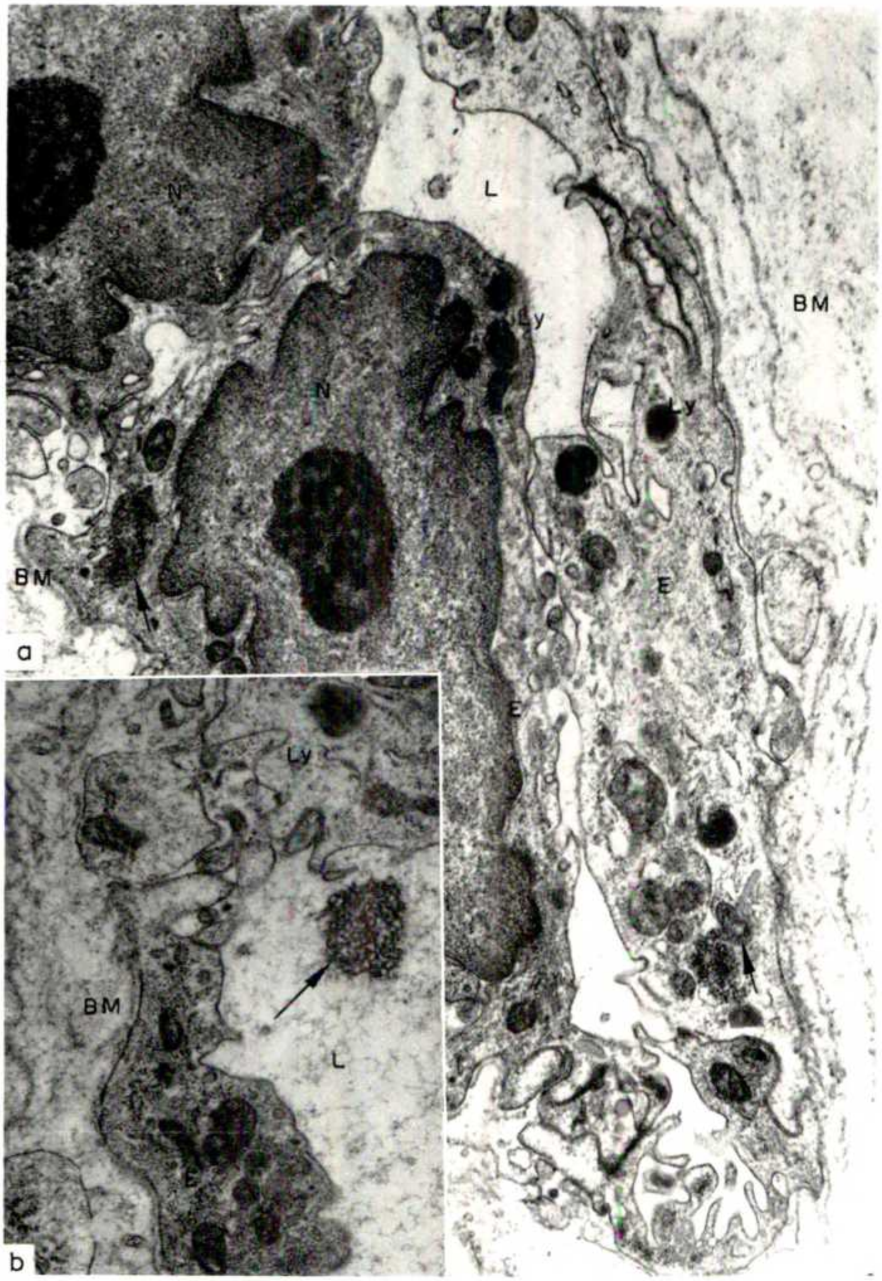

FIGURE 1. Dermal capillary. (a) Three inclusions (arrow) are seen in different endothelial cells (E), and (b) free in the lumen (L). N, nucleus; BM, basement membrane; Ly, lysosomes. (a) × 1200, (b) × 20,000.

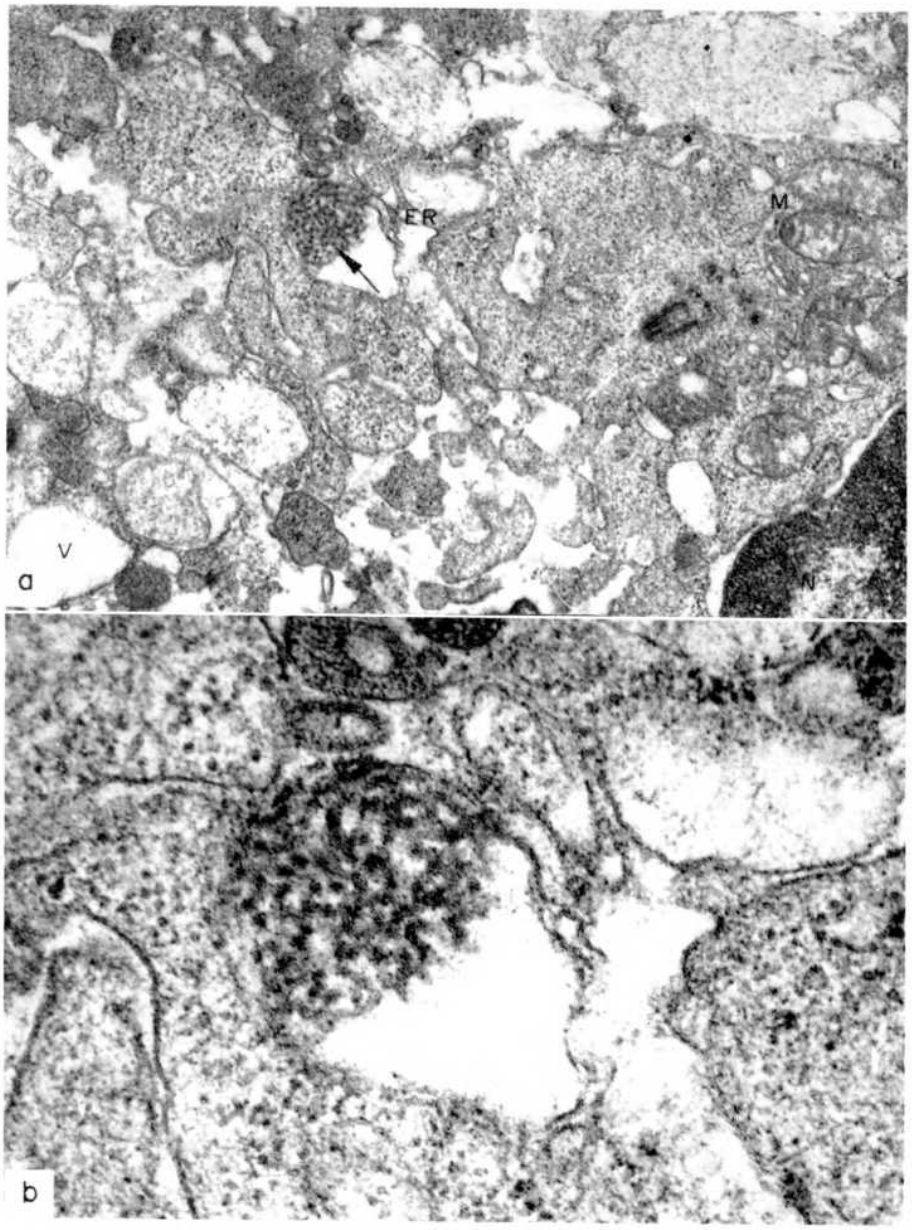

FIGURE 2. Inclusions composed of tubular structures (arrow) in enlarged endoplasmic reticulum (ER) of a macrophage (a), also at higher magnification (b). M, mitochondrium; V, vacuole; N, nucleus. (a) × 2,5000, (b) × 80,000.

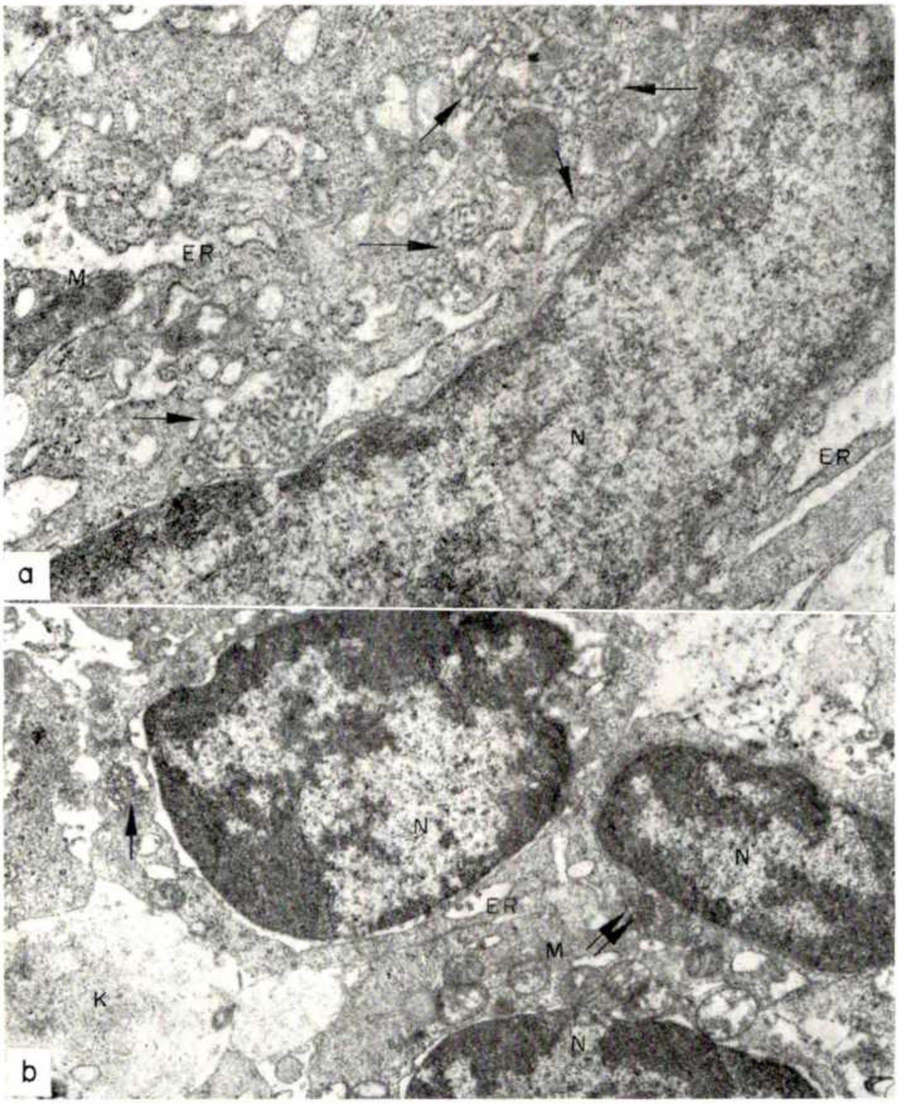

FIGURE 3. Tubular structures (arrow) in the enlarged endoplasmic reticulum (ER) near the nucleus (N) of a fibroblast (a) and of lymphocytes of the dermal inflammatory infiltrate (b). M, mitochondria; double arrow, inclusion in the perinuclear cisterna; K, Corium. (a) × 20,000, (b) × 12,000.

However, in two cases similar, but not identical, structures were found in the nucleus of lymphocytes (Haustein, 1973c). In one specimen of CDLE the tubular structures appeared to be floating freely in the lumen of the blood vessel (Fig. 1). In two other specimens of SLE the structures, partly enveloped by a small cytoplasmic border and by a membrane, were found in the cytoplasmic fragments of a necrobiotic zone of the corium near the macrophages and polymorphonuclear leukocytes (Fig. 5).

Morphological characteristics of tubular structures

The inclusions measured up to 2·8 μm in diameter and were seen as interwoven, anastomosing

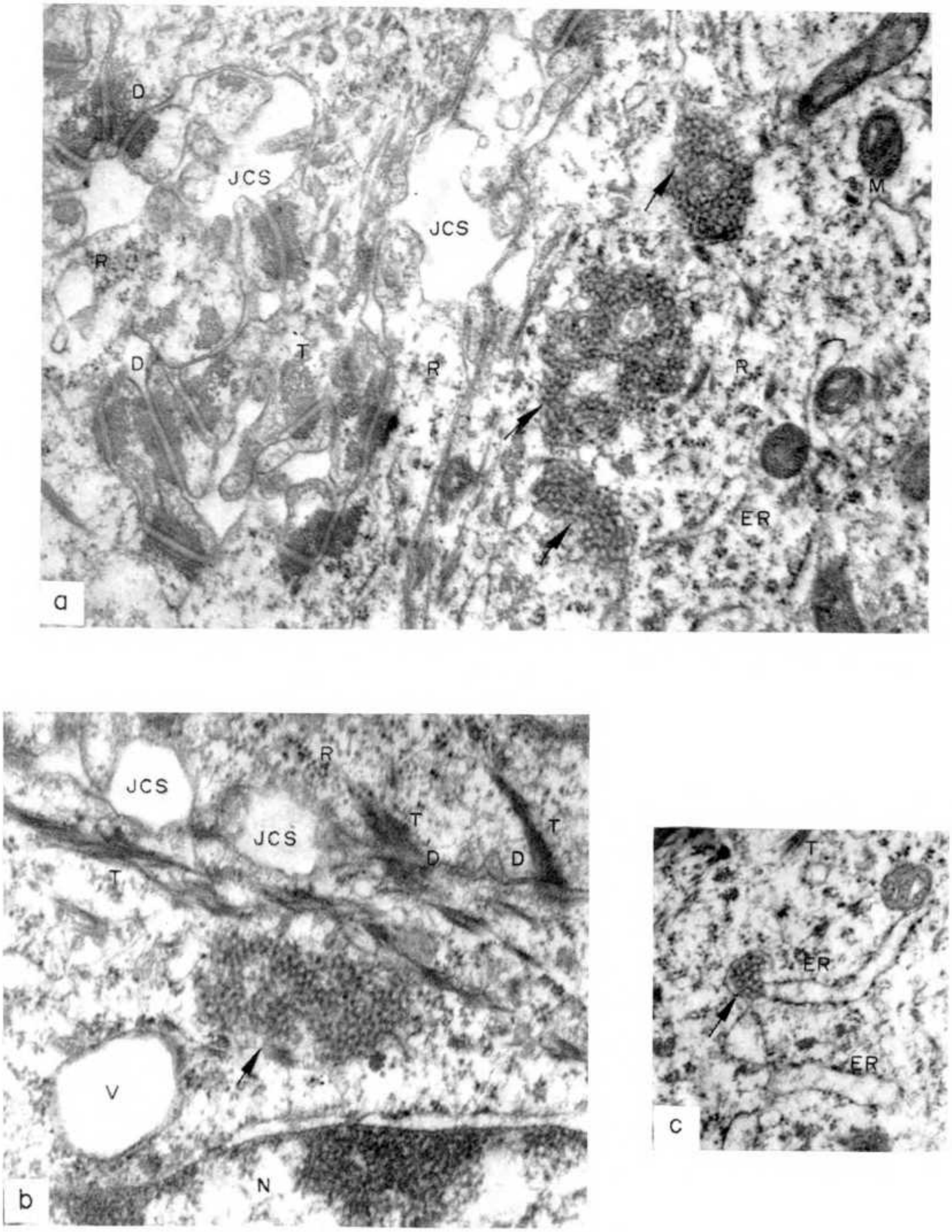

FIGURE 4. Keratinocytes of the deeper layers of the epidermis. Tubular structures (arrow) partly free in the cytoplasm (a and b), partly within the endoplasmic reticulum (ER) (a and c), partly near the nucleus (N) (b). R, ribosomes; M, mitochondria; D, desmosomes; T, tonofilaments; ICS, intercellular space; V, vacuole. (a) × 30,000, (b) × 40,000, (c) × 30,000.

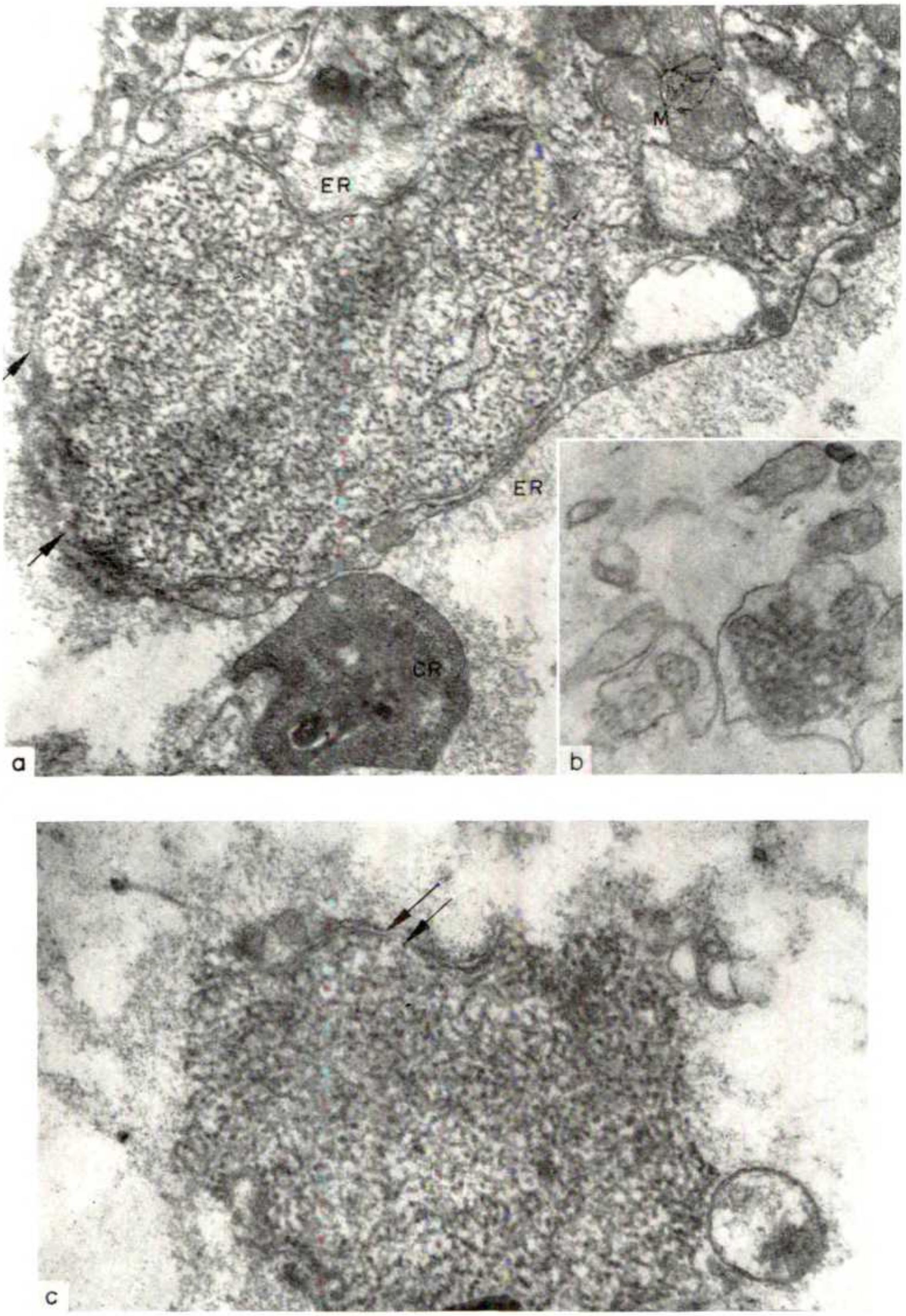

FIGURE 5. A large inclusion of tubular structures in the endoplasmic reticulum (ER) of a macrophage within a necrobiotic area. Arrow, breakage of the membrane of the ER; M, mitochondria in various stages of degeneration; CR, rest of a necrobiotic cell (a). Processes of a cell with tubular structures within a necrobiotic zone (b). Tubular structures free in the necrobiotic area of the corium. Double arrow, remains of the membrane of the ER (c). (a) × 30,000, (b) × 40,000, (c) × 40,000.

reticulate tubular structures. The tubules measured approximately 20–28 nm in diameter in transverse, oblique or longitudinal section and up to 90 nm in length. The centre was often less electron dense than the periphery, represented by an outer layer approximately 5 nm in thickness, whereas the electron lucent core varied from 10–18 nm in diameter. In other instances the tubular structures were without lumen. The tubular unit was round in cross-section. The uranyl acetate procedure distinctly improved the contrast.

Pathological changes of 'host' cells

The affected fibroblasts, histiocytes and macrophages were rich in various organelles: mitochondria, partly degenerate, enlarged endoplasmic reticulum, golgi complexes, lysosomes and pinocytotic vesicles, suggesting enhanced metabolic activity. The endothelial cells with tubular structures contained an increased number of multi-vesiculated and diffusely dense bodies (lysosomes), multiple pinocytotic vesicles, occasionally vacuoles, myelin figures and densely packed intracellular filaments. The mitochondria were often enlarged and vacuolized. In specimens from SLE the basement membranes of the blood vessels were irregularly thickened and contained a number of immune-complex deposits. The number of pericytes was increased. The occasionally involved basal cells and keratinocytes showed degenerative changes. The lymphocytes mostly did not show any signs of cell damage.

The usual characteristic features of LE, at the dermo-epidermal junction, with necrobiosis of the basal cells, and in the corium, with nuclear and cellular breakdown, formation of fibrillar bodies, as well as basement membrane changes and deposits of immune complexes (wire loop and fibrin), are described elsewhere (Grishman & Churg, 1970; Haustein, 1973a, b; Tuffanelli, Kay & Fukuyama, 1969).

Correlation between frequency of tubular structures and clinical forms, acuity of lesions and previous treatment

As shown in Table 1 we found such tubular structures in the affected skin in nine out of nine SLE, and in twenty-two out of twenty-eight of CDLE patients. The normal skin was involved in six out of six specimens of SLE examined, and in six out of eighteen of CDLE, predominantly in severe, subacute and disseminated forms. They occurred both in involved and normal skin independently of previous treatment with steroids. Sometimes several tissue blocks were examined before an area with inclusions was found. In some instances two or three inclusions were present in one cell. In Table 2 the previous treatment duration of CDLE and the age of the examined lesion are summarized and correlated with the frequency with which tubular structures were found. The data appear to indicate a correlation with the degree of injury to the tissue. In acute lesions in patients with disease of short duration we found that these inclusions were more frequent and were more densely packed. On the other hand there was no relationship with previous treatment. In the controls no inclusions were observed.

DISCUSSION

The tubular structures illustrated here have been described in 'collagen' and autoimmune diseases (Bloodworth & Shelp, 1970; Hashimoto *et al.*, 1971; Norton, Velayos & Robison, 1970; Prunieras *et al.*, 1972), in various apparently unrelated renal diseases (Garancis *et al.*, 1971; Hurd *et al.*, 1971; Tisher *et al.*, 1971), in viral infections (Haas & Yunis, 1970; Howe & Morgan, 1969), and in leukaemias, lymphomas and malignant tumours (Uzman *et al.*, 1971). They have also been observed in cell cultures and transplanted cell lines from patients with lymphomas and other lympho-proliferative disorders (Chandra, 1968) and in various animal species (Uzman *et al.*, 1971). Because of their wide-

TABLE 1. Tubular structures in affected and clinically normal skin of LE patients in relation to previous treatment with steroids

Diagnosis	No.	Female	Male	Previous treatment				Tubular structures				Not examined	
								Present		Absent			
SLE	9	9	0	Glucocorticoids	6		Affected skin	6		–		–	
							Normal skin		4		–		2
				No treatment		3	Affected skin	3		–		–	
							Normal skin		2		–		1
Total	9	9	0		6	3		9	6	–	–	–	3
CDLE	3	2	1	Glucocorticoids	3		Affected skin	3		–		–	
Subacute							Normal skin		1		1		1
Disseminated	8	2	6	Glucocorticoids	2		Affected skin	2		–		–	
							Normal skin		1		–		1
				No treatment		6	Affected skin	6		–		–	
							Normal skin		3		1		2
Localized	17	12	5	Glucocorticoids	4		Affected skin	2		2		–	
							Normal skin		0		2		2
				No treatment		13	Affected skin	9		4		–	
							Normal skin		1		8		4
Total	28	16	12		9	19		22	6	6	12	–	10
Combined total	37	25	12		15	22		31	12	6	12	–	13

spread occurrence it is not clear whether these tubular inclusions represent aetiological agents, nonpathogenic passenger organisms or cellular reaction products, secondary to the disease.

Although the observed inclusions resemble ultrastructurally the nucleocapsides of myxo- or paramyxoviruses (Györkey & Sinkovics, 1971; Hurd *et al.*, 1971), corona- or onco-RNA-viruses (Sinkovics, 1970) there are differences which argue against their viral nature. The nucleoprotein strands of the known paramyxoviruses are smaller (15 to 18 nm in diameter) and often lie intranuclearly or free in the cytoplasm in parallel, loosely arranged, nonbranching, uncoiled arrays without limiting membranes of endoplasmic reticulum (Morgan & Howe, 1968; Nakai, Shand & Howatson, 1969; Pincus *et al.*, 1970). In paramyxovirus infections, neither budding from the wall of the endo-

TABLE 2. Relation between the tubular structures in affected skin in patients with CDLE, the previous treatment and the duration of the disease, and the duration of the lesions examined

Previous treatment	Tubular structures		Duration of lesion	
			< 6 months	> 6 months
Glucocorticoids (4)	Positive	2	2	0
	Negative	2	1	1
Chloroquine (6)	Positive	4	3	1
	Negative	2	0	2
No treatment (7)	Positive	5	3	2
	Negative	2	1	1
Total (17)	Positive	11	8	3
	Negative	6	2	4

plasmic reticulum (Figs. 2 and 3) nor from the nuclear membranes (Hashimoto *et al.*, 1971; Haustein, 1972) have been described. We have never seen tubular structures budding from or entering the cell. Schmitt *et al.* (1971) interpreted the areas of locally modified density of the cell membrane as sites of maturation of virus elements in fibroblasts of CDLE. The intranuclear size of tubular structures analogous to the cytoplasmic aspect and the formation of syncytial cells, both characteristics of paramyxovirus infected cells, were recently found in LE by Hashimoto & Chandler (1972) and by Nieland *et al.* (1972).

Several attempts at virus isolation in tissue cultures as well as co-cultivation and cell-fusion have yielded no positive results (Feorino, Heirholzer & Norton, 1970; Hashimoto *et al.*, 1971; Philipps, 1971; Pincus *et al.*, 1970). However Hashimoto & Chandler (1972) found large inclusions of identical tubular structures in tissue culture cell lines. Moolten, Clark & Mihalyfi (1970) managed to isolate virus-like agents and to identify them by serological and immunological methods, but not by electron microscopy. The negative results of immunofluorescence assays with various hyperimmune antiviral sera (Norton, 1969; Pincus *et al.*, 1970) may be explained; in that the unenveloped nucleocapsides may lack both the specific viral protein coat and the envelope derived from the host cell plasma membrane. The enhanced antibody titres in SLE sera to a large number of viruses (Evans, 1971; Hashimoto *et al.*, 1971; Hollinger *et al.*, 1971; Kalliomäki & Halonen, 1972; Norton, 1969; Philipps & Christian, 1970) as well as to other antigens suggest nonspecific rather than a specific immunological hyperreactivity. But the occurrence of antibodies against double stranded RNA (both synthetic and viral) in SLE sera supports the possibility that a viral form is the natural immunogen (Lamon & Benett, 1970; Tatal, Steinburg & Daley, 1971). Perhaps a lysogenic or defective RNA-virus (Huang & Baltimore, 1970) may contribute to the immunological imbalance and to the formation of 'nuclear' autoantigens by RNA-dependent DNA-polymerase (Temin & Mizutami, 1970). The RNA-nature of the tubular structures is suggested by the fact that uranylacetate increased and ribonuclease abolished the tubular density (Györkey & Sinkovics, 1971; Hashimoto *et al.*, 1971).

Several authors assume that the tubular structures may represent cellular material, e.g. altered nuclear material or immune complexes (Haas & Yunis, 1970) released the damaged cells and phagocytosed by endothelial cells (Hurd *et al.*, 1971). This hypothesis seems unlikely since the rough-surfaced endoplasmic reticulum is not primarily involved in phagocytosis, no surrounding phagosome membrane is visible, and different cell types are affected. Particularly in CDLE, there is no evidence of any direct cytotoxic effect attributable to viruses, although lysosomal inclusion structures were abundant in endothelial cells as a minor sign of vascular damage in LE and dermatomyositis (Hashimoto & Thompson, 1970; Hashimoto *et al.*, 1971; Ohashi, 1969). However, the structures free in the blood vessel lumina (Fig. 1; Hashimoto & Chandler, 1972; Hashimoto & Thompson, 1970) and in the necrobiotic dermal tissue of SLE (Fig. 5) may be regarded as secondary to release from degenerating cells. It is unlikely that the tubular structures reflect a single response or regenerative product of a variety of insults (Haas & Yunis, 1970; Norton *et al.*, 1970; Pincus *et al.*, 1970; Uzman *et al.*, 1971).

As shown in Tables 1 and 2 previous treatment with steroids does not influence the formation of tubular structures; a finding in agreement with those of Garancis *et al.* (1971). Chloroquine, which may induce lysosomal changes in skin blood vessels (Ohashi, 1969) also does not influence the findings. In CDLE we observed the inclusions more frequently and more densely packed in patients with a short history and acute, active dermal lesions (Table 2). Our findings agree with those of Hashimoto & Thompson (1970) who described them in 100% of new lesions, in 70% of older ones and never in very old scarred lesions. Similarly, Norton (1969) found inclusions in various organs of patients with SLE; Bloodworth & Shelp (1970) and Garancis *et al.* (1971) in lupus nephritis; and Hashimoto *et al.* (1971) and Norton *et al.* (1970) in dermatomyositis.

In the affected areas and in normal skin we found the tubular structures in a higher percentage of patients with SLE than with CDLE (Table 1). Recently Nieland *et al.* (1972) have described tubular structures in the normal skin of nine patients with SLE; Prunieras *et al.* (1971) in patients with atypical dermatomyositis; and Garancis *et al.* (1971) in those with LE-nephritis. These findings in normal skin indicate that the inclusions have no immediate role in producing dermal lesions, although they are the earliest detectable changes. Moreover, the investigation in patients with CDLE (Haustein, 1973d) of other organs such as the kidney, lymph node, bone marrow and the peripheral blood leukocytes has also given positive findings. We interpret these facts as evidence of the generalization of the pathological process in CDLE, particularly in the severe, subacute and disseminated forms. Alternatively they may indicate latent SLE.

There is a possible relationship between the presence of the inclusions and immune complex formation, since the clinically normal skin in SLE frequently shows positive fluorescence and ultrastructural changes at the dermo-epidermal junction (Burnham & Fine, 1971; Grishman & Churg, 1970; Tufanelli *et al.*, 1969). The tubular structures have been found in keratinocytes (Fig. 4; Schmitt *et al.*, 1971) and in hydropically degenerated basal cells. It is possible that ultraviolet irradiation allows the spread of the virus in epidermal cells and provokes the LE-specific dermal lesions, perhaps by alteration of the lysosomal membranes.

We suggest that the expression of the disease is dependent on individual reactivity of a genetically determined immune system, stimulated by an as yet undetermined virus infection. In view of the findings of Hashimoto & Chandler (1972) we suggest that the tubular structures represent an unidentified and unusual virus, rather than a cellular reaction product related to such virus infection. They occur in tissue culture cell lines independent of the immunological influences and defence mechanisms of the host. The question whether they are aetiological or nonpathogenic 'passenger' agents is still unanswered; since they occur with high frequency they may be used as a diagnostic aid in patients with suspected LE.

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An attempt to identify the nature of immunoglobulin-complement deposits in the skin in lupus erythematosus

TH. VAN JOOST

Department of Dermatology (Chairman: Prof. Dr R.H. Cormane), University of Amsterdam,
Binnengasthuis, The Netherlands

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SUMMARY

An attempt was made to verify whether DNA is located in the Ig deposits present in LE skin.

No DNA could be detected in the deposits after incubation of lesional skin sections of five patients with LE (two SLE and three DLE) with antisera directed against DNA and UV-DNA, after preliminary ultraviolet irradiation of the sections.

Duplicated skin sections previously subjected to an acid elution also gave negative results.

Immunoglobulin-complement (Ig-C) deposits occur in the skin of patients with LE (SLE and DLE) in the absence in the serum of circulating antibodies reacting with the junctional zone of skin (Cormane, 1964; Beutner *et al.*, 1965; Kalsbeek, 1968).

Acid elution techniques have been applied in LE in renal and skin biopsies by several authors. In this field the evidence was used that antigen–antibody complexes can dissociate at acid pH 3·0–3·5. With regard to the skin the results can be summarized as follows: Thivolet, Beyvin & Le Mot (1972) used a micro-acid elution technique of Feltkamp & Boode (1970), performed on cryostat sections of lesional skin. The authors reported finding nuclear antibodies in the eluate of one SLE patient of a group consisting of five SLE and six DLE patients. Antibodies directed against the junctional zone of rabbit skin were found in the eluate of three cases (one SLE and two DLE) of the same group of patients.

Using a macro-acid elution technique of Koffler, Schur & Kunkel (1967), performed on large strips of skin (3–30 cm), Landry & Sams (1972) demonstrated antibodies reacting with nuclei in the skin eluates of three patients with SLE. In two of these cases a strong antibody was also demonstrable, reactive with the basement membrane zone of epithelial tissues, e.g. normal human skin, rabbit oesophagus and guinea-pig oesophagus.

From these observations it seems possible that Ig deposits in the SLE skin contain at least two different antibodies.

One might also expect different antigens to be present in LE skin, reactive with circulating antibodies not yet demonstrated. The possible nature of the deposits in the skin in LE has been discussed by Cormane & van Joost (1973).

Since their discovery (Seligmann, 1957) antibodies to DNA have been repeatedly demonstrated in sera of SLE patients. It has subsequently been found (Barbu, Seligmann & Joly, 1960; Stollar & Levine, 1961) that the majority of SLE sera appear to react more strongly with the heat-denatured DNA (single strand) than with native DNA (double strand).

It is of interest that ultraviolet irradiation of the DNA molecule, normally a very weak antigen makes the molecule strongly antigenic.

Levine *et al.* (1966) found antibodies with high titres against UV-induced DNA (UV-DNA) in the serum of rabbits, after injection of UV irradiated DNA into these rabbits.

By means of the immunofluorescent method (double layer technique), using an antiserum directed against UV-DNA, Tan & Stoughton (1969) and Ten Veen & Lucas (1970) were able to demonstrate changes in DNA (UV-DNA) in the UV-irradiated skin of mice.

Considering the fact that in the skin in LE the deposits may consist of DNA–anti DNA complexes, an attempt was made by means of the indirect immunofluorescent method (IIF) to confirm the presence of DNA in these deposits.

MATERIALS

Biopsies of lesional skin (3 mm) were obtained from two SLE patients with circulating antinuclear antibodies and from three DLE patients without circulating antinuclear antibodies. In all these biopsies Ig deposits could be demonstrated.

METHODS

In this study, antisera directed against DNA and UV-DNA were used. Duplicated sections were irradiated with UV light prior to incubation with the antisera. In addition an attempt was made to make the antigen (DNA ?) accessible for the specific antisera by removing the immunoglobulins by micro-elution at acid pH (Feltkamp & Boode, 1970).

For this purpose the following procedures were carried out: some cryostat sections of 4 μm thickness were mounted side by side on a glass slide. The sections were not fixed and were subsequently covered with 0·2 ml (0·02 M) citric buffer at pH 3·2, and incubated in a humid atmosphere at 4°C for one night. The next day, after the acid citrate solution had been carefully removed, the sections were subjected to the same procedure and after irradiation were incubated respectively with anti-DNA serum and anti-UV-DNA serum.

For the ultraviolet irradiation of the skin sections on glass slides a Philips germicidal lamp was used; this lamp which emits predominantly light of 253·17 nm was placed 10 cm above the sections for 10 min.

Reagents

For the detection of DNA an antiserum was used, obtained by immunization of rabbits with normal denatured calf thymus DNA (single strand), complexed with equivalent amounts of MBSA.

In the IIF performance test this antiserum reacted with nuclei of tissue at serum dilutions between 120 and 240.

For the detection of UV-DNA a rabbit anti-UV-DNA serum was used. This antiserum was prepared according to the method described by Levine *et al.* (1966).

In the IIF performance test, this antiserum reacted with nuclei of irradiated tissue at serum dilutions between 1000 and 2000. In unirradiated skin no nuclear staining could be detected with this antiserum.

In the present study the anti-DNA serum was used in a dilution of 1:10 and the anti-UV-DNA serum in a dilution of 1 : 25.

For the second step (IIF method) a horse anti-rabbit Ig/FTC serum was used. For the DIF method to demonstrate Ig deposits in the lesional skin sections a horse-anti-human Ig/FTC serum was used.

Characteristics of FTC–protein conjugates

Horse-anti-rabbit Ig/FTC: protein concentration, 7·4 mg/ml; Mol. F/P ratio—2·5 (dilution 1/50). Horse-anti-human Ig/FTC: protein concentration, 10·6 mg/ml; Mol. F/P ratio—2·8 (dilution 1/45).

Antisera and conjugates used for this study were prepared in the Central Laboratories of the Netherlands Red Cross Blood Tranfusion Service, 125 Plesmanlaan, Amsterdam.

Microscope

A fluorescent microscope was used with a xenon lamp (XBO 150) as light source. Dichroic mirror, 495 nm exciting filters: 2 × KP 490, 1 mm in combination with TAL 475. Barrier filter: OG 515, 3 mm.

RESULTS

The results (Table 1) show reactivity of the used antisera in all the five cases examined, with nuclear material present in the nuclei of epidermis and dermis, but not with the deposits.

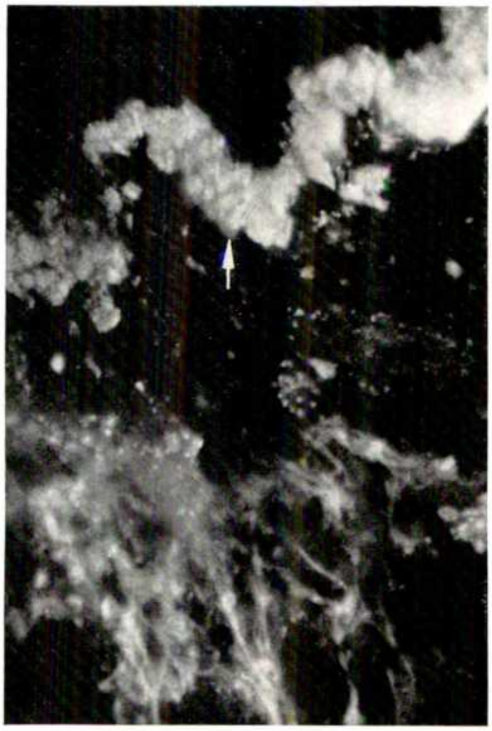

FIGURE 1. DIF pattern in case of lesional skin (DLE). Blue narrow-band excitation. Objective 50, water immersion, ocular 10. A granular staining of deposits is predominantly present in the dermo-epidermal region. (Horse-anti-human Ig/FIC.)

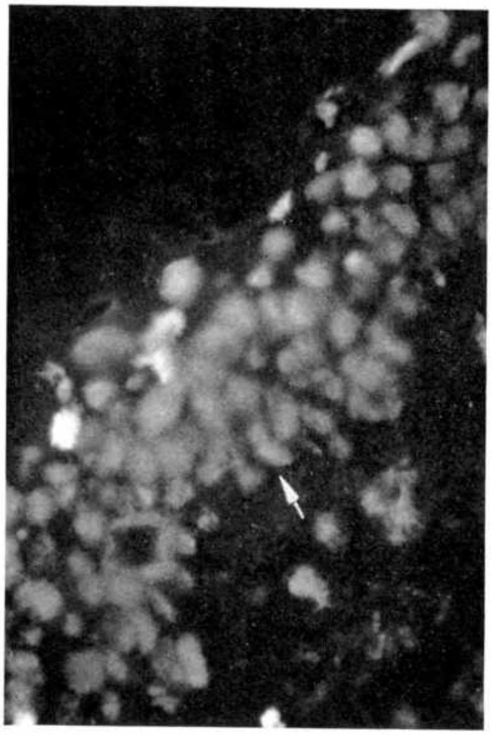

FIGURE 2. IIF pattern of a duplicate skin section (DLE, same case as Fig. 1) after acid elution and subsequent incubation with anti-UV-DNA serum after ultraviolet irradiation of the skin section. Blue narrow-band excitation. Objective 50, water immersion, ocular 10. No UV-DNA could be shown at the localization of Ig deposits in the junctional zone. (The nuclear staining in the epidermis and dermis is present, but less conspicuous than in sections on which no acid elution was performed.)

TABLE 1. Results of DIF and IIF tests on the occurrence of DNA in the deposits in the lesional skin of five patients with LE (two SLE, three, DLE)

No. of patients	Results DIF		Acid elution method	UV irradiation	IIF using HaRlg/FTC in the 2nd step	Results IIF	
	DEJ*	Nucleus				DEJ	Nucleus
2SLE, 3DLE	+	–			anti-DNA	–	+
2SLE, 3DLE	+	–		applied	anti-UV-DNA	–	+
2SLE, 3DLE	+	–	applied		anti-DNA	–	+
2SLE, 3DLE	+	–	applied	applied	anti-UV-DNA	–	+

*DEJ; dermo-epidermal junction.

After acid elution ('to remove the masking effect of possible nuclear antibodies') and subsequent incubation with anti-DNA serum and anti-UV-DNA serum after UV irradiation, no DNA or UV-DNA could be noted at other localizations than the nuclei.

It is of note that in duplicate skin sections after acid elution DIF-test showed a diminishing or abolishing of the staining pattern of the Ig deposits, thus indicating the removal of immunoglobulins. If, however, phosphate buffered saline (PBS) was used instead of the acid citric buffer, no impairment of staining of the deposits was observed.

DISCUSSION

To get a better understanding of IF findings in LE skin it is important to know the nature of the deposits demonstrable. In the cases of DLE it was thought possible that all the antinuclear antibodies were trapped by immune-complexes so that they were not demonstrable in the serum.

The results of this study are not in favour of the assumption that the deposits contain DNA. Our findings are in agreement with observations of Tan & Kunkel (1966), who did not find abolition of demonstrable staining the DIF-method 'stippled staining pattern' at the dermo-epidermal junction, after pretreatment of lesional SLE skin sections with desoxyribonuclease (DNAse). It therefore seems useful to establish whether we are dealing here with antigens different from DNA, such as RNA, nucleoproteins and soluble nuclear components, responsible for the generation of the Ig-C deposits. However, the negative findings could be the result of removal of the antigen from the skin sections by the acid procedure, or alteration or presumed antigenic determinants by the method applied.

Besides an explanation based on an immunological reaction the possibility of a non-immunological mechanism should also be taken into consideration. It is conceivable that patient's own immunoglobulins together with complement, in the absence of antigens, are deposited in the dermal and junctional regions of the skin in LE, due to physico-chemical changes promoting aggregation of serum proteins at these sites. Investigations on antigens other than DNA are currently in progress.

ACKNOWLEDGMENTS

I am indebted to Dr T.E.W.Feltkamp (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam) for placing the anti-DNA serum and the anti-UV-DNA serum at my disposal.

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Sebum excretion in hypopituitarism

S.K.GOOLAMALI, J.L.BURTON, AND SAM SHUSTER

Wellcome Laboratories for Research into Skin Disease, University Department of Dermatology,
Newcastle-upon-Tyne

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SUMMARY

The sebum excretion rate (SER) was measured in thirty-one patients with hypopituitarism of diverse aetiology, and the mean SER was found to be greatly decreased compared with control patients of the same age. The decrease was apparent even in patients who were receiving replacement therapy with hydrocortisone, thyroxine and testosterone. These findings confirm the importance of the pituitary gland in maintaining sebaceous activity in man, and the nature of the pituitary sebotrophic factor is discussed.

Little is known of the influence of the pituitary on the human sebaceous gland. Burton *et al.* (1972a) measured sebum excretion in pituitary hyperfunction due to acromegaly and we here report the effect of hypopituitarism on sebum excretion in man.

METHODS

The sebum excretion rate (SER) was measured from the forehead skin of fifteen female and sixteen male patients with pituitary hypofunction by the method of Strauss & Pochi (1961) as modified by Cunliffe & Shuster (1969). The patients, aged 14–64 years, had hypopituitarism of diverse aetiology but the extent of the pituitary deficiency had been established in every case by appropriate investigations. Three of the thirty-one patients had an apparently isolated deficiency of somatotrophin (GH), two had isolated gonadotrophin deficiency, and the remainder had a mixed deficiency of pituitary hormones due either to pituitary tumour, surgical hypophysectomy, head injury, Sheehan's syndrome or previous tuberculous meningitis. Eight of the latter twenty-six patients also had diabetes insipidus. No replacement therapy had been given to the five patients with isolated deficiency of somatotrophin or gonadotrophin at the time of the SER measurement, but the other twenty-six patients were receiving replacement therapy with oral hydrocortisone, thyroxine and antidiuretic therapy as necessary. In addition, the ten male patients who complained of impotence were treated with testosterone, usually methyltestosterone (Testoral) 40 mg sublingually daily.

The SER was also measured in seventy male and ninety-three female control subjects aged 10–70 years. These consisted of patients attending the skin out-patient department for a variety of localized dermatoses such as verrucae and tinea pedis, and their healthy relatives.

RESULTS

The mean SER in the fifteen female patients with pituitary hypofunction was 0·21 ± SE 0·05 μg/cm²/min, and this was significantly less than the mean SER of 0·42 ± SE 0·08 μg/cm²/min observed in the sixteen male patients ($P<0·05$).

The SERs in the thirty-one patients are shown compared with the control SERs in Figs. 1 and 2.

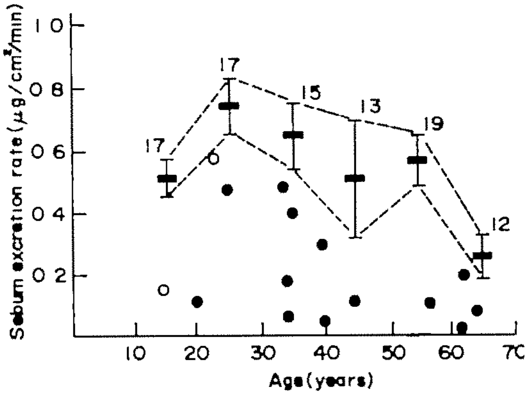

FIGURE 1. Sebum excretion rates in fifteen female patients with hypopituitarism or isolated growth hormone deficiency, and in ninety-three female controls. Connected dashed lines represent controls (mean ± SE); ●, hypopituitarism; ○, isolated GH deficiency.

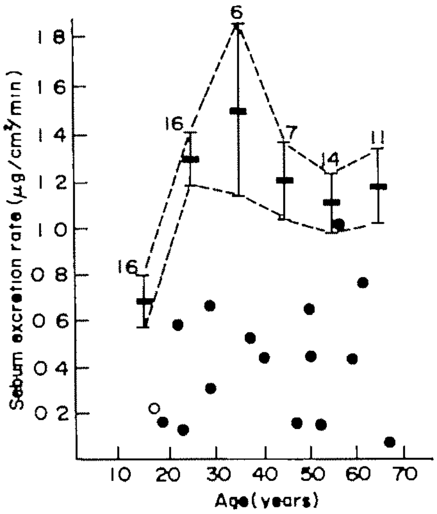

FIGURE 2. Sebum excretion rates in sixteen male patients with hypopituitarism or isolated growth hormone deficiency, and in seventy male controls. Connected dashed lines represent controls (mean ± SE); ●, hypopituitarism; ○, isolated GH deficiency.

The SER for each patient was subtracted from the mean SER of the controls of the corresponding age and sex. The mean difference for the female patients was 0·34 ± SE 0·09 μg/cm²/min and for the males 0·74 ± SE 0·07 μg/cm²/min, and both these decreases were highly significant ($P<0·001$).

Owing to the heterogeneous nature of the group it was impossible to analyse the results further with regard to individual hormone deficiencies.

DISCUSSION

The results show clearly that our patients with hypopituitarism had a sebum excretion rate which was considerably reduced compared with control subjects of the same age and sex. This is compatible with previous studies in the rat which have shown the pituitary gland to be necessary for the maintenance of normal sebaceous function (Ebling, Ebling & Skinner, 1969; Ebling *et al.*, 1970; Nikkari & Valavaara, 1969; Thody & Shuster, 1970a, b). An interesting difference between the human and the rat data however is the degree to which sebum secretion is decreased by pituitary hypofunction. In the hypophysectomized rat the SER does not appear to fall below about one-third of the level in the intact animal (Thody & Shuster, personal communication), whereas a much greater decrease was observed in some of our patients with hypopituitarism. This species difference may be related to the greater importance of body hair in the rat. The reduction in SER caused by hypophysectomy in the human is presumably due to the removal of a pituitary sebotrophic hormone. Little is known of the effect of the various pituitary hormones on the human sebaceous gland, but corticotrophin (ACTH), thyrotrophin (TSH) and gonadotrophin are known to be sebotrophic in the rat (Thody & Shuster, 1970a, b, 1972b), their effect being mediated by the corresponding target endocrine gland in each case.

Another important point which emerges from the present study is that the SER was considerably reduced despite the fact that the patients were receiving replacement therapy with hydrocortisone, thyroxine or testosterone in doses which appeared to be clinically adequate. There are two possible explanations for this observation. Either the human pituitary gland produces a potent sebotrophic hormone other than corticotrophin (ACTH), thyrotrophin (TSH) and gonadotrophin, or else these hormones are required in a permissive role to allow hydrocortisone, thyroxine or testosterone to stimulate sebum secretion. Further work will be needed to clarify this, but it is unlikely that gonadotrophins are required to allow testosterone to exert its sebotrophic effect since Nikkari & Valavaara (1969) and Thody and Shuster (1970a) have shown that in the hypophysectomized rat the sebaceous glands respond normally to physiological doses of testosterone. The contrary findings of Ebling *et al.* (1969) appear to be due to the insensitivity of hair lipid measurements (Shuster & Thody, 1973).

The possibility that the pituitary produces another potent sebotrophic hormone must therefore be considered. This is unlikely to be somatotrophin (GH) because although in a previous study we found the SER was increased in acromegaly and decreased after pituitary ablation the correlation between SER and serum GH levels was poor (Burton *et al.*, 1972a). Furthermore, the sebotrophic activity of GH is relatively low in the rat (Nikkari & Valavaara, 1969; Ebling *et al.*, 1969; Thody & Shuster, 1970b). Our present observation that the SER was reduced in three patients with apparently isolated GH deficiency might support the suggestion that GH is an important sebotrophic hormone in man, but it must be pointed out that levels of other potential sebotrophic hormones, such as melanocyte-stimulating hormone (MSH), were not measured in these patients. This hormone must be considered since our previous work suggests the possibility that a powerful human pituitary sebotrophic hormone exists which is under inhibitory control by the hypothalamus (Thody & Shuster, 1972a; Burton & Shuster, 1970; Burton *et al.*, 1972b). Both MSH and prolactin are under inhibitory control but prolactin has no effect on sebum excretion in the hypophysectomized (Thody & Shuster, 1970b; Nikkari & Valavaara, 1970) or intact rat (Thody & Shuster, unpublished) whereas MSH is sebotrophic (Thody & Shuster, 1972a). Furthermore, Thody & Shuster (1972a) found that removal of the neurointermediate lobe of the rat pituitary reduced sebum secretion almost as much as total hypophysectomy. Thus the evidence from the rat suggests that an MSH-like hormone may be the previously undefined pituitary sebotrophic factor, and the decreased MSH in patients with hypopituitarism might explain the low SER which occurs even in patients receiving replacement therapy with thyroxine, cortisol and androgen. Before applying these findings from the rat to the clinical situation, however, further work is required in man to test the sebotrophic potential of MSH, prolactin and GH.

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Meibomian gland secretion in the red eyes of rosacea

*C.C.CORY, WENDY HINKS, J.L.BURTON AND SAM SHUSTER

Wellcome Laboratories for Research into Skin Diseases, University Department of Dermatology,
Newcastle-upon-Tyne and *Department of Ophthalmology, Royal Victoria Infirmary, Newcastle-upon-Tyne

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SUMMARY

Methods are described for the measurement of the quantity and the composition of Meibomian gland secretion. Using these methods, we are unable to detect any difference in Meibomian gland lipid between control subjects and patients with red eyes associated with rosacea.

The cause of the blepharitis and dilated conjunctival vessels of rosacea is unknown. The Meibomian glands of the eyelid are modified sebaceous glands and the pilo-sebaceous unit is affected in rosacea. It has been suggested that free fatty acids are irritant (Kellum 1968; Ray & Kellum, 1971) and it seemed possible that the red eyes of rosacea might be due to increased fatty acids in the Meibomian gland secretion. We therefore studied the composition and quantity of Meibomian gland lipid in this condition.

METHODS

We studied eleven patients with rosacea and red eyes, who were aged 32 to 70 years, and seven control subjects aged 23 to 40 years who had errors of refraction but had no skin disease or conjunctivitis. Only one patient (subject 9) was receiving tetracycline at the time of the study.

Measurement of Meibomian gland function

The contents of the Meibomian gland were expressed from each lower eyelid in every patient by squeezing the lid between a sterile metal spatula and the thumb. This procedure was painful, but the discomfort was lessened, though not eliminated, by the installation of local anaesthetic (Amethocaine) into the eye 10 min before the collection procedure. The lipid material which was expressed from the orifices of the glands was collected on the spatula and transferred to small absorbent ether washed cellulose pledgets which were placed into clean 75 ml beakers. Thereafter the lipid was extracted from the pledget into weighed metal cups with three successive washes with 25 ml Analar ether. After evaporation of the ether, the lipid was dried at 37°C, equilibrated to room temperature and then weighed. The details of this extraction procedure were as described by Cunliffe & Shuster (1969) for the extraction of sebum.

Composition of Meibomian gland secretion

The composition of the lipid was determined by a thin-layer chromatographic technique as follows.

Reprint requests should be addressed to Professor Shuster.

The lipid samples from both eyelids were pooled in each patient, dissolved in a small volume of ether and spotted onto 20 × 20 cm glass plates coated with silica Gel G (0·25 mm thick). Both normal and rosacea samples were spotted onto the same plate. The plates were run in a multiple development solvent system of decreasing polarity; the later less polar solvents being allowed to run further up the plate than the earlier more polar ones. The solvent system used was as follows: (1) hexane: diethyl ether : glacial acetic acid (80 : 20 : 1), developed to 7·8 cm; (2) hexane : diethyl ether (95 : 5), developed to 15·5 cm; (3) hexane, developed to 15·5 cm.

After burning of any hydrocarbons at 120°C for 20 min the plates were sprayed with sodium dichromate (0·25%) in 20% aqueous sulphuric acid. Charring was achieved by heating at 160°C for 2 h. The charred spots were identified by reference to standard compounds developed on the same plates. The plates were then scanned under a densitometer and the peaks from the resultant traces weighed and expressed as a percentage of the whole trace.

RESULTS

The quantity of lipid expressed from each eyelid and its percentage composition are shown in

TABLE 1. Melbomian gland lipid in rosacea with conjunctivitis and control patients

Subject	Sex	Lipid (mg)			Percentage composition				
		Right	Left	Total	Monoesters	Diesters	Triglycerides	Free fatty acids	Free sterols
Conjunctivitis patients									
1	M	3·36	2·47	5·83	39·6	24·3	5·6	17·8	13·1
2	M	1·46	2·46	3·92	53·3	22·9	3·2	10·7	9·4
3	M	1·24	1·27	2·51	39·9	19·8	5·0	14·2	20·0
4	F	0·61	0·83	1·44	54·5	12·5	8·2	14·1	10·9
5	F	0·80	0·75	1·56	72·5	20·3	0	2·1	5·2
6	F	0·94	0·51	1·45	79·2	13·9	0	4·7	2·0
7	F	0·70	0·26	1·56	68·6	15·1	0	11·7	4·6
8	F	0·24	0·38	0·62	—	—	—	—	—
*9	F	0·53	0·27	0·70	63·8	10·9	7·2	9·1	9·0
10	F	0·36	0·80	1·16	61·8	12·6	10·2	9·3	6·6
11	F	1·15	1·17	2·32	62·2	15·6	9·3	7·6	5·4
Mean		1·04	1·04	2·07	59·5	16·8	4·87	10.13	8·6
SE		0·26	0·23	0·47	4·10	1·49	1·24	1·47	1·64
Control subjects									
1	M	0·85	1·34	2·19	62·9	12·4	10.0	7·9	6·9
2	M	0·36	0·25	0·61	78·4	18·9	0	0	0
3	M	1·87	1·70	3·57	58·0	19·1	0	18·5	4·3
4	F	0·92	0·61	1·53	66·0	17·2	5·0	3·5	8·2
5	F	0·92	0·91	1·83	57·3	22·5	8·9	8·9	11·4
6	F	—	—	4·44	60·3	18·2	0	15·8	5·6
7	F	—	—	1·34	66·6	15·1	0	18·3	0
Mean		0.98	0·96	2·21	64·2	17·6	2·1	10·4	5·2
SE		0·25	0·26	0·50	2·7	1·2	2·8	2·8	1·6

*Receiving tetracycline.

Table 1. There was considerable variation in the quantity of lipid collected in different patients, but there was good agreement between the quantity collected from the right and left lower lids in each patient. The mean weight of lipid collected from each rosacea patient was 2·1 ± SE 0·5 mg and this did not differ significantly from the mean value of 2·2 ± 0·5 mg in the controls.

The Meibomian gland secretion consisted mainly of sterol and wax monoesters (about 60%) with some diesters (about 17%) and smaller proportions of free fatty acids, free sterols and triglycerides.

There was no significant difference in percentage composition of the lipid between rosacea patients and controls, and in particular the proportion of free fatty acids was virtually identical in the two groups.

DISCUSSION

Our results suggest that the lipid secreted by the Meibomian glands in rosacea is normal in quantity and composition and the red eyes seen in this condition are not due to an increase in the total free fatty acid content. The present observations do not of course exclude an increase of a specific irritant such as a prostaglandin.

Previous studies of the composition of Meibomian gland secretion have given conflicting results. Linton, Curnow & Riley (1961) reported that paper chromatography of pooled Meibomian secretions from 539 subjects revealed mainly neutral fat, with some phospholipid and an unidentified lipid, but they could find no cholesterol or free fatty acids. Keith (1967) studied fifty patients with blepharitis, three of whom had rosacea, and he found a high proportion of wax and cholesterol esters, with a moderate content of cholesterol and low free fatty acids, but virtually no triglycerides. Ehlers (1965) reported similar findings. Our present study supports the latter two authors, since we found the secretion consisted of esters, with small quantities of free fatty acids and free sterol, and triglycerides varying from zero to 10%.

The good agreement between the amount of lipid we collected from the glands in the right and left eyelids in the individual, suggests that our technique of collection and extraction provides a reproducible index of Meibomian gland function. This method might usefully be applied to the study of Meibomian gland function and its relationship to other ophthalmic problems.

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Alteration of subcultured keratinocytes to a keratinizing epithelium

F.W.BAUER AND R.M.DE GROOD

Department of Dermatology (Head Prof. Dr J.W.H. Mali), University of Nijmegen, Javastraat 104,
Nijmegen, The Netherlands

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SUMMARY

Subcultured epithelial cells from guinea-pig ear skin were aggregated by means of nucleoprotein. After subsequent culturing under organ-culture conditions, a more or less organized epithelium which formed a new stratum corneum was reconstituted.

Cell cultures have been used, since Loeb's original observations (1897), in many fields of cell biology and experimental medicine. In dermatology attempts have been made to use suspensions of epithelial cells for grafting procedures (Pruniéras, Delescluse & Régnier, 1969). Unfortunately, grafting was successful only with cells from primary cultures; attempts to use cells from secondary and later subcultures in order to profit from the cell gain have proved unsuccessful. Pruniéras was unable to account for the failure of such experiments, although he rejected the obvious possibility of the keratinocyte population being replaced by fibroblasts during successive subculturing.

The major difficulty in this kind of work is that the factors governing the choice between mitosis or differentiation of a keratinocyte are almost totally obscure. We have, therefore, selected a parameter which seems likely to be linked with such a choice (the tendency to aggregation of the cells in culture) and have investigated the influence of a number of chemical and physical variables on this parameter.

Our preliminary results are presented.

MATERIALS AND METHODS

Epidermal cell suspensions were prepared from adult guinea-pig ear skin (Cruickshank, Cooper & Hooper, 1960; Pruniéras, 1965). Small pieces of skin, cut from the ear with a razor blade, were incubated at 37°C in a solution of 0·15% trypsin (DIFCO 1:250) in Hank's solution, without magnesium and calcium ions, the skin floating at the surface of this solution. After 30 min the pieces were transferred to a Petri dish containing the culture fluid. Then the epidermis was removed from the dermis by gently scraping the surface. The epithelial fragments were sucked into a pipette until a suspension of keratinocytes was obtained.

Falcon tubes VN 30 were filled with 1·8 ml culture medium (M 199) and 0·2 ml foetal calf serum. To the culture fluid was added 200 units of penicillin G (sodium penicillin G, Mycofarm, The Netherlands) and 100 μg streptomycin (streptomycin sulphate, Mycofarm, The Netherlands) per ml culture fluid. The suspension contained approximately 10^6 cells/ml. The incubation temperature was 37° C. The cells were allowed to adhere; after 24–48 h the unattached cells were discarded, and fresh medium was added. The cells were subcultured at appropriate intervals.

Deoxynucleoprotein was isolated from calf thymus by the method of Chargaff (1956), and resuspended in fifty times the volume of water used by this worker.

EXPERIMENTAL PROCEDURE

The cells of a confluent monolayer of the sixth to the twentieth subculture were trypsinized in the ordinary way (trypsin, Difco 1:250) (Pruniéras 1965). After removal of the trypsin, the cells were allowed to settle in one corner of the culture vessel after the addition of 1 ml medium M 199, and the culture was inspected at intervals for the appearance of one or more 'clots' resulting from spontaneous aggregation. Various factors were investigated which might influence aggregation of the cells. These included pH (6 to 7·5); temperature (4, 20 and 37°C); serum concentration (5% and 10%); the presence of trypsin inhibitor (0·1 mg in 1 ml culture medium, 3 × crystallized from soybean, Calbiochem, U.S.A.) and the addition of nucleoprotein.

In the case of nucleoprotein, additional experiments were carried out as follows: 0·1 ml of dilute nucleoprotein preparation was added to the culture vessel immediately after removal of the trypsin. After a few moments 1 ml of medium (M 199) was added, and the aggregated cells were transferred to a millipore filter (pore diameter 0·45 μm). This was maintained under organ culture conditions for 3 or 4 days. Culture conditions, fixation procedures and histology were as described by Bauer & Kuyper (1972); some sections were subsequently incubated with DNAase in a concentration able to destain Feulgen stained nuclei.

RESULTS

In those cultures which succeeded, the cells at first grew very slowly. After one month, the primary

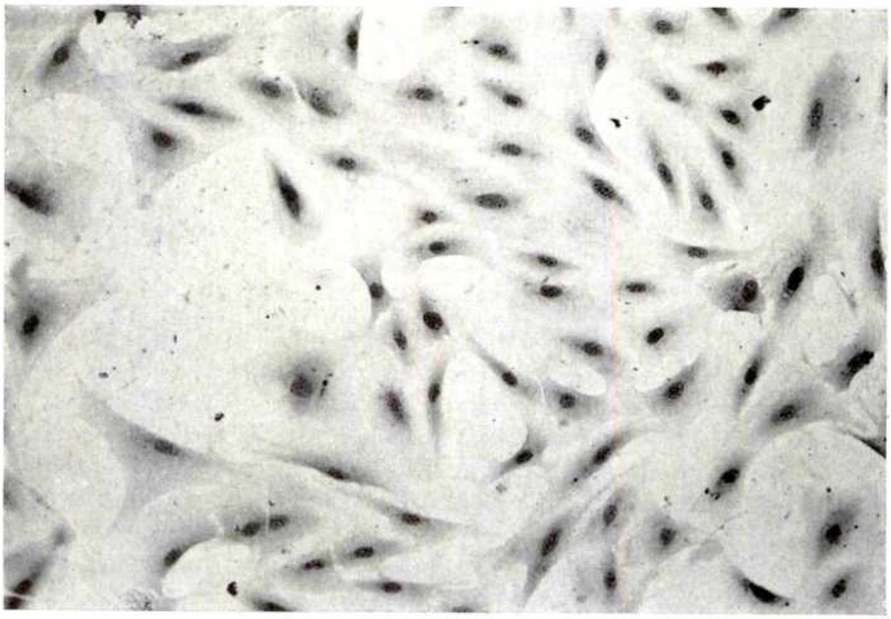

FIGURE 1. Subculture of guinea-pig keratinocytes. Note the fibroblast-like appearance (H & E, × 102).

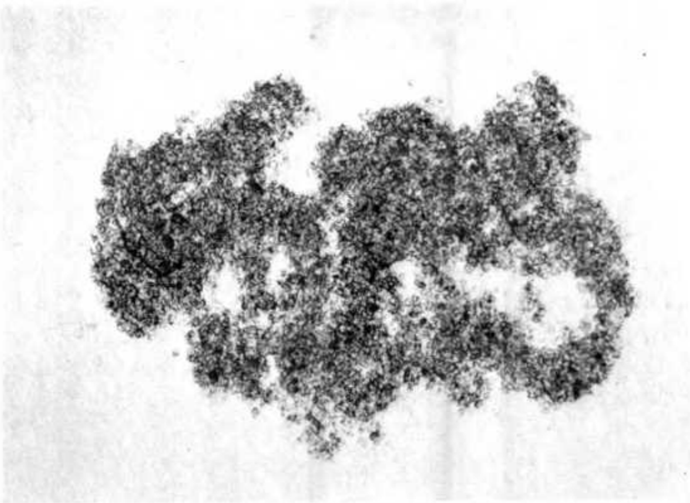

FIGURE 2. Aggregation of epithelial cells after the addition of nucleoprotein (×25).

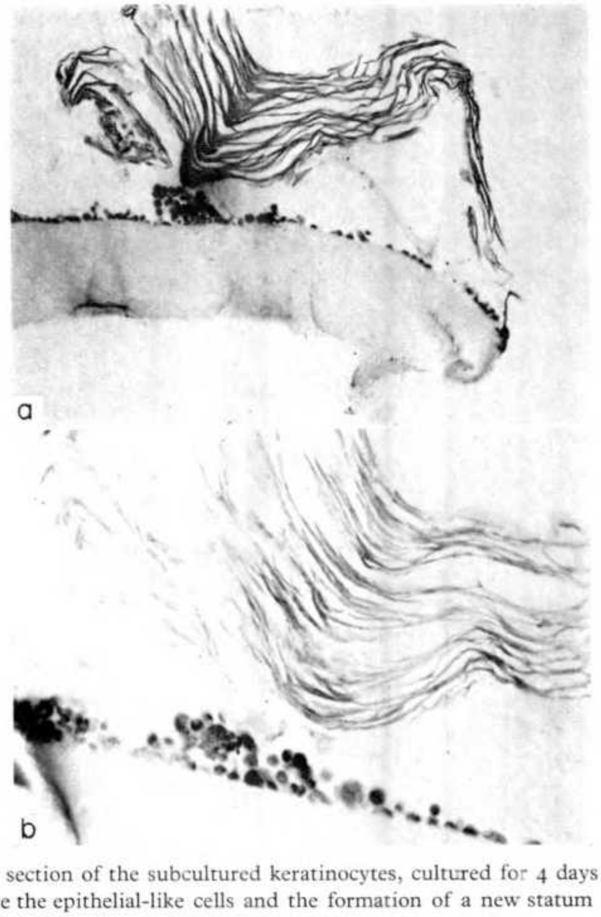

FIGURE 3. Cross section of the subcultured keratinocytes, cultured for 4 days in a multilayered arrangement. Note the epithelial-like cells and the formation of a new statum corneum (H & E; a, × 102; b, × 250).

culture could be subcultured into two vessels. The intervals between subcultures gradually became shorter: 14 days after three or four passages and about 7 days after ten passages. Fig. 1 shows cells from typical monolayer cultures.

It was found that neither pH, temperature, concentration of serum nor the addition of trypsin inhibitor had any significant effect on aggregation. The addition of nucleoprotein, however, markedly enhanced the formation of clots; a typical aggregate is shown in Fig. 2.

Fig. 3 shows sections through the cultured cells grown for 4 days under organ culture conditions on millipore filters. As can be seen, a more or less organized epithelial tissue is present, which has synthesized a new stratum corneum. Incubation with DNAase had no effect on this stratum corneum, eliminating the possibility that this was an artefact resulting from the added nucleoprotein. The stratum corneum of these sections was strongly birefringent. The occasional spontaneously occurring aggregates (probably the result of nucleoprotein released by the trypsin from disrupted cells) showed essentially the same characteristics as described above.

DISCUSSION

These experiments have established that an 'aggregate' forms in which the micro-environment of an individual cell might be expected closely to resemble that encountered in living epidermis. Our results clearly suggest that this is indeed the 'trigger' which initiates the process of differentiation. The reason for this remains open to speculation, but the creation of a concentration gradient with respect to the nutrients in the culture medium is an obvious possibility.

The reconstitution of a viable 'epidermis' is demonstrated by the appearance in these experiments of a keratin layer (Fig. 3). This keratin layer is produced by the 'epidermis', since no fragments of keratin were ever observed during the passages prior to reconstitution.

It is clear that this kind of investigation can be important as a model system to gain insight into differentiation processes at a molecular level. Experiments are now in progress directed towards obtaining a better organization of the 'tissue'; in addition electron microscopy may make it possible to demonstrate the formation of desmosomes between the epithelial cells.

ACKNOWLEDGMENTS

We are indebted to Professor Ch.M.A.Kuyper for giving valuable advice and for reading the manuscript.

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A hand-held friction meter

J.S.COMAISH, P.R.H.HARBOROW,* AND D.A.HOFMAN*

Department of Dermatology, Royal Victoria Infirmary, Newcastle-upon-Tyne NE1 4LP, and
* Design Unit, Department of Mechanical Engineering, Newcastle University,
Newcastle-upon-Tyne

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SUMMARY

A portable hand-held friction meter has been devised which will measure the coefficient of friction between a reference material and skin at any body site regardless of position. The reference surface can be made of any of a number of materials, such as steel, nylon, PTFE, leather or even fabrics. The effects of disease and topically applied medicaments on the skin are being studied quantitatively with this machine, which may also have industrial applications.

Skin is a material at once accessible and amenable to precise measurement; in particular its simplest physical properties can be measured and recorded provided suitable instruments are to hand. One physical function of basic importance is the friction we depend upon for gripping and locomotion. Excess of friction force produces erosions and blisters in everyone, and more readily so in pathological conditions such as epidermolysis bullosa or pemphigus. Clearly, measurements of friction could be the basis for useful research in such diseases.

In our early work we found ourselves hampered by the cumbersome nature of the machines currently available for measuring friction. We needed a portable, preferably hand-held device, which could be used in clinic and ward, and at any site regardless of position. Surprisingly, no such instrument existed, either in the world of medicine or of engineering. This was all the more astonishing when the importance of friction is considered in relation to industry—brake shoes, car tyres, clothing and footwear, being just a few of the many articles and materials whose coefficient of friction profoundly affects their everyday performance.

The Newcastle friction meter has been developed to satisfy the requirement for a small, robust, easily operated instrument that will give comparative readings of the coefficient of dynamic friction between any two materials, one material being the test material and the other a standard reference material. For simplicity the friction meter applies a constant contact load and operates at constant speed, so that the coefficient of friction is measured at a specific surface pressure and rubbing velocity. In the application for which it was designed, it is required that the coefficient of friction be measured on various parts of the body, necessitating that the instrument is hand-held and able to operate in any attitude.

DESIGN FEATURES

The friction meter is a small, completely self-contained instrument, which is fully portable requiring only periodic re-charging of the storage batteries at the mains (Figs. 1 and 2).

In contrast to other friction measuring devices, the friction head does not rub with a reciprocating motion, but is an annular ring rotating at constant velocity. The rotating head eliminates errors due to the elastic behaviour of measured materials (such as skin) and of directional surface structure—for example machining marks. Since there is continuous movement transient stick-slip does not occur and a steady reading is obtained.

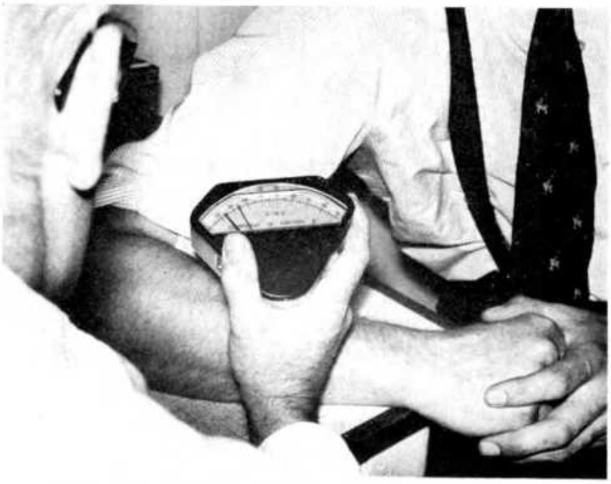

FIGURE 1. The Newcastle friction meter in use.

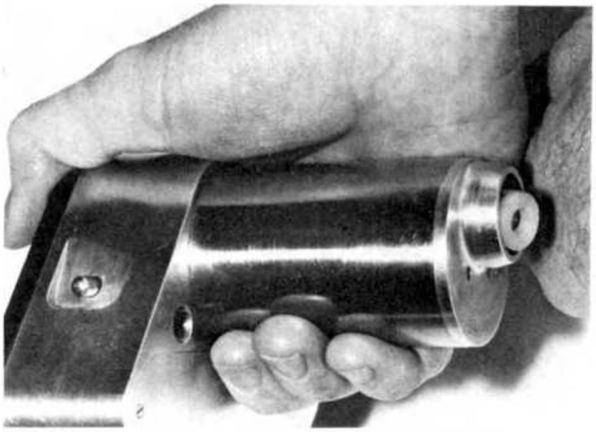

FIGURE 2. Newcastle friction meter—rotating ring.

The rotating ring can be made of any one of a number of reference materials, such as steel, nylon, PTFE or even of fabrics or animal and vegetable tissue. The ring is sprung onto a friction head which is fully articulated and allows the annular ring to make square contact with the test surface. Constant surface loading is maintained by an adjustable constant force spring which loads the friction head spindle, which is free to move axially. The instrument itself can be supported on the surface to be measured by resting the outer tube on the surface, the friction head protruding through this tube. All moving parts are fully counterbalanced and supported in anti-friction bearings. The friction head drive is provided by a miniature precision electric motor driving through a reduction gear head and a sliding coupling to the friction head spindle. The drive unit is suspended from self aligning instrument ball bearings, and restrained from rotating by a calibrated torsion spring. The torque reaction from the drive being equal to the friction torque, a direct calibration of the instrument can be carried out knowing the specific loading and annular rubbing pad friction diameter. The indicating pointer gives a direct reading on the dial in the instrument head and is fitted to the drive carrier.

The meter has been found to give extremely reproducible results when tested in practice. Since the specific loading and rubbing speed are low, temperature effects at the friction surface can be neglected.

Skin friction meter specification

Friction head loading	200 g
Friction head rotational speed	150 rev/min
Maximum variation in rotational speed	+10% to −0%
Rubbing pad diameter	15 mm
Rubbing pad friction radius	5·5 mm
Range of measurement of coefficient of friction	0–1·8
Maximum error in indicated coefficient of friction	3% full scale deflection
Power source—two rechargeable nickel cadmium cells	
Instrument weight	790 g
Overall length	124 mm

The friction meter (which we propose to call the Newcastle Friction Meter)* has been in continuous use over the past 6 months. It has enabled us to make quantitative observations on the skin of patients with a variety of conditions, particularly in those where friction trauma plays an important role in producing the symptoms and signs of their disease. We hope that clinicians will emulate this quantitative approach to dermatology and explore methods of measuring other physical properties of skin, such as elasticity, reflectivity and toughness—in fact any of the physical properties by virtue of which skin is of significance to the intact organism.

* Patent applied for.

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How accurate is the visual assessment of basal carcinoma margins?

ERNST EPSTEIN

Department of Dermatology, University of California Medical Center,
San Francisco, California 94122, U.S.A.

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SUMMARY

This study evaluated the accuracy of visual determination of tumour margins in basal cell carcinomas. Using a 2·5 × binocular magnifier, the apparent tumour margin was marked pre-operatively by light electrodesiccation. Following excision, the electrodesiccation mark was compared to the actual tumour edge by microscopy of step level sections. It was possible to delineate the tumour margins with an accuracy of 1 mm in 94% of a selected series of 101 basal cell carcinomas. These findings support the view that a 2 mm margin of normal tissue is adequate in the surgical treatment of most basal cell carcinomas.

Complete tumour removal is the goal of surgical treatment of basal cell carcinoma. To assure complete removal, it is standard practice to include an 'adequate' margin of normal tissue in the excision. What constitutes an adequate margin? Textbooks (Ochsner & DeBakey, 1959; Freeman & Knox, 1967) generally recommend minimum margins of 5–10 mm, adding that each situation must be evaluated individually. Experience has led me to the conclusion that such 5–10 mm margins are unnecessarily large in excising the basal cell carcinomas encountered in a dermatological practice, and for some years I have used a 2 mm minimum margin. In my hands the 2 mm margin has proved satisfactory, having resulted in a cure rate of over 98% in more than 200 surgically excised basal cell carcinomas followed for over 2 years. Such a 2 mm margin is quite different from the traditional 5 to 10 mm margins, and raises the question: what is the basis for the varying recommendations as to 'adequate' margins? It appears that all previous recommendations as to 'adequate' margins are essentially opinions, as a search of the literature failed to uncover any objective studies on this point.

In fact, the phrase 'margin of normal skin' is incorrect, for no one wishes to excise normal tissue. What is implied is normal appearing skin which may harbour carcinoma undetected by the physician's eye. The sacrifice of normal skin is an admission of our inability to determine exactly the extent of basal cell carcinoma. A rational decision as to the optimal margin of normal tissue to excise requires knowledge of the accuracy of visual assessment of the margins of a basal cell carcinoma. This report compares the actual tumour margins, as microscopically determined from sections, with the visually estimated margins delineated pre-operatively by electrodesiccation.

MATERIALS AND METHODS

During the 1-year period beginning May 1970 I marked the visually determined margins of basal cell

Reprint requests to: Ernst Epstein, 36 South El Camino Real, San Mateo, California 94401, U.S.A.

carcinomas diagnosed in my dermatological practice. Before local anaesthesia, and with the aid of a binocular loupe magnifying 2·5 diameters, the apparent margins of the basal cell tumour were outlined with a felt-tipped marking pen. The proposed excision line was then marked with a different coloured pen allowing minimum* margins of 1·5–2 mm between this excision line and the previously marked tumour outline. Following local lidocaine anaesthesia, the previously marked tumour outline was lightly electrodesiccated using a monopolar current from a commercial electrosurgical unit equipped with a sharply pointed electrode. The tumour was then excised as a single intact specimen, placed in 10% formalin, and submitted to the pathology department of Mills Memorial Hospital, San Mateo, California.

The pathology department of Mills Memorial Hospital routinely examines step-level sections of skin cancers for marginal extension of neoplasm. After fixation the specimen is divided at right angles to its axis into segments 1·5 mm in thickness. Two or more full face sections are cut from the surface of each paraffin embedded segment and then mounted on slides and stained. This technique enables the pathologist to examine representative sections of the specimen with a maximum tissue interval of 1·5 mm. With small specimens (5 mm diameter or less) the procedure is modified in that the block is not subdivided, the technician mounting approximately 10 sections at intervals as he cuts into the block; consequently the tissue interval between sections is less than 0·5 mm.

During this study, the pathologists were asked to determine and report in writing whether the electrodesiccated margins coincided with the microscopic margin within 1 mm, or whether they were more than 1 mm apart. In addition, I independently examined all slides. The visual assessment of margins was considered successful when both observers concluded that the electrodesiccated site and the actual tumour margin were within 1 mm. If either concluded that the electrodesiccated margin failed to correspond within 1 mm of the microscopic margin, the case was considered a failure.

In analysing the data, it proved useful to correlate the histological findings with the ability visually to demarcate the borders of the lesions. I adopted a simplified classification of basal cell tumours into three categories: (1) nodular, (2) superficial, and (3) infiltrating. Not all neoplasms fitted into this scheme; if a tumour had features of two or more types, it was classified according to the dominant characteristic. A tumour was classified nodular when it was made up of large masses of tumour cells. Superficial epithelioma is a standard term describing those tumours showing buds and irregular proliferation of epithelioma attached to the under surface of the epidermis. Infiltrating basal cell epithelioma referred to those tumours characterized by more or less thin strands of basal cell carcinoma extending into the surrounding tissue. The term infiltrating was chosen as it accurately describes a histological picture. Alternate terms for infiltrating epitheliomas such as 'morphoea-like' are unsatisfactory as they refer to a gross clinical appearance possessed only by a proportion of infiltrating basal cell epitheliomas.

In a significant number of basal cell carcinomas excised during the study period, it was impossible to delineate the tumour margins pre-operatively by electrodesiccation because the tumour had been destroyed during an earlier biopsy. I customarily remove small possibly malignant growths superficially using scissors or curettage. This is a practical approach since if the lesion proves benign, no further treatment is necessary. If it is malignant, the patient returns for surgical excision, but unfortunately all gross tumour has been removed, rendering both the marking by electrodesiccation, and the microscopic assessment of actual tumour margins, impossible. In this situation, the excision was performed to leave a 2 mm minimum margin beyond the border of the biopsy wound.

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* The entire surgical border does not always represent a minimum margin. If the tumour is excised as an ellipse, the borders along the long axis of the ellipse will considerably exceed the 'minimum' 2 mm margins used to determine the surgical borders of the short axis of the ellipse. Somewhat less than half of the tumours in this series were excised as an ellipse with the minimum margins delineating the short axis. In the majority, the cancer was excised as a round disc, and in these the entire margin was a minimum margin.

RESULTS

During a 1-year period 131 basal cell carcinomas in ninety-three patients were diagnosed. All but six cases were ultimately treated by surgical excision. The overall results are summarized in Table 1, with pre-operatively marked neoplasms being classed as successes when the electrodesiccation margin was within 1 mm of the actual tumour margin, and failures when more than 1 mm separated them. In 93% of the 101 cancers marked pre-operatively, the microscopic assessment revealed the visual assessment of margins to be accurate within 1 mm (Group A).

TABLE 1. Summary of visual margin assessment study of 131 basal cell carcinomas diagnosed in ninety-three patients

	No. of tumours	Diameter of tumours (mm)		No. of tumours (%)		
		Mean	Range	Nodular	Superficial	Infiltrating
Group A Successful (within 1 mm) determination of actual tumour margins	94	8	3–20	68 (73)	20 (21)	6 (6)
Group B Failure (more than 1 mm discrepancy) in determining actual tumour margins	7	13	9–18	2 (29)	3 (43)	2 (28)
Group C Margins not attempted	24*	7†	4–17	11 (46)	9 (38)	4 (16)
Group D Therapy elsewhere	6‡	14	12–18	1 (16)	0	5 (84)

* Margins were not attempted in twenty-four tumours for the following reasons: tumour destroyed by prior biopsy, twenty lesions; tumour clinically considered benign, two lesions; forgot to electrodesiccate margin, two lesions.

† Estimate of tumour size in twenty lesions destroyed by preceding biopsy was derived by measuring the size of the crust at the biopsy site.

‡ One patient refused surgery, the other five had large and/or previously treated infiltrating tumours of the nose and were referred for chemosurgical treatment.

Group B summarizes the findings in the seven cancers in which the actual margins differed by more than 1 mm from the electrodesiccated margins. In six of these seven failures the tumour extended beyond the electrodesiccated margin, while in the seventh the electrodesiccated margin was several millimeters outside the tumour. In the six where the tumour extended more than 1 mm beyond the electrodesiccated margin, the surgical margin was free of tumour in four, and in these four the tumour was less than 3 mm beyond the electrodesiccated margin. In two patients the tumour extended to the surgical margin. A repeat excision in one of these two patients showed only a healing wound without demonstrable residual cancer. In the second patient, an elderly man, it was decided to observe the site periodically rather than re-excise, since it has been shown that even with tumour involvement of surgical margins, less than 50% recur (Gooding, White & Yatsahashi, 1965).

There were significant differences between the success and failure group of neoplasms. The tumours in the failure group tended to be larger (mean diameter 13 mm) than those successfully demarcated (mean diameter 8 mm). The higher percentage of superficial and infiltrating basal cell carcinomas found in the failure group is to be expected, for it is obviously more difficult to determine the margins in these two types of cancer.

Group C consists of the twenty-four lesions in which pre-operative electrodesiccation was not performed. In twenty the surface tumour had been destroyed by prior biopsy. These were subsequently excised with a 2 mm margin beyond the crust covering the biopsy site. In these twenty lesions excised after prior biopsy had destroyed the visible tumour, sections of the excised tissue revealed eleven free of residual tumour, while nine showed residual tumour. In all but one of these nine with residual tumour, the surgical margins were free of tumour. Since the tumour was completely destroyed by preliminary biopsy in eleven out of twenty, and in nineteen out of twenty the surgical margins were free of residual tumour, it is likely that if clinical assessment of margins had been performed in these lesions, the results would have been comparable to the series where margins were attempted.

While the surgical margins were free of tumour in nineteen of the twenty lesions grossly destroyed by preliminary biopsy, in one patient, the tumour did extend to all margins. This patient, a woman aged 54 presented with a 4 mm roughened area of the left cheek which appeared to be an actinic keratosis. Biopsy by curette removal of the lesion revealed infiltrating basal cell carcinoma. When surgical excision in the usual conservative way showed extension to all margins, a second more generous excision was performed well into the completely normal appearing surrounding skin. This second excision was also unsuccessful, and when a third excision demonstrated further extension at one margin, the patient was referred for microscopically controlled excision by the Mohs chemosurgery technique.

Four intact epitheliomas escaped electrodesiccation. Two were clinically believed benign while in the other two I forgot to perform the pre-operative electrodesiccation. In all four of these intact lesions, the surgical margins were free of tumour.

125 of the 131 neoplasms encountered during the study were treated by conservative surgical excision. In all cases 2 mm minimum margins of normal tissue were employed. In those lesions destroyed by preceding biopsy, it was assumed that the biopsy site represented the extent of the tumour. Excision of these 125 lesions resulted in tumour free margins in 122 (98%).

Six lesions were treated elsewhere (Group D). Five were infiltrating epitheliomas of the nose referred for chemosurgical treatment with the Mohs technique. Two of the infiltrating carcinomas of the nose had prior treatment elsewhere (one with X-ray, the other with electrodesiccation) with much scar formation. The other three were women with fairly large (over 10 mm) infiltrating lesions; it was felt that in these three patients Mohs chemosurgery offered the best chance of cure with the least sacrifice of normal tissue. The sixth patient refused treatment; his 7 mm nodular cancer was removed some months later by his general surgeon while the patient was undergoing cholecystectomy. While there was a preponderance of infiltrating epitheliomas of the nose referred for treatment elsewhere, it should be noted that three other infiltrating epitheliomas of the nose were encountered in this series and were successfully excised with accurate pre-operative delineation of margins within 1 mm.

DISCUSSION

It proved possible to delineate the tumour margins pre-operatively with an accuracy of 1 mm in 94% of 101 basal cell carcinomas. This suggests that the traditional textbook recommendations of 5 to 10 mm minimum margins of normal tissue are unnecessary in the primary treatment of most basal cell carcinomas. Use of a 2 mm margin of normal appearing tissue resulted in surgical margins free of tumour in 122 out of 125 carcinomas so treated. While the absence of recurrence over a 5-year follow-up period is the ultimate criterion of successful treatment, it is generally accepted that surgical excision is adequate and recurrences are rare when the excision margins are free of tumour (Pascal *et al.*, 1968).

If 2 mm margins are adequate in over 95% of early basal cell carcinomas, why are much larger margins recommended as standard treatment? In this series more generous margins would have been successful in producing surgical margins free of neoplasm in only two out of three of the cancers where the tumour involved the margins. In the third patient, the previously described woman with the cheek lesion, even 15 mm margins of normal tissue would have been inadequate! The recommendations of 5 to 10 mm margins of normal tissue usually originate from plastic surgeons and are based on their experience (Macomber, Wang and Sullivan, 1959; Hayes, 1962; Grabb *et al.*, 1969). The spectrum of basal cell carcinomas treated by plastic surgeons differs markedly from those presenting to the dermatologist. While 76% of the carcinomas in the present series were under 1 cm in diameter, only 45% of the tumours reported by Grabb *et al.* (1969) were smaller than 1 cm. The large, over 3 cm diameter lesion which occurs significantly in a plastic surgery practice, was not once encountered in my study.

Plastic surgeons not only treat large lesions; recurrent tumours also form a significant part of their practice. While less than 2% of the carcinomas in my series had had previous treatment, 16% of the basal cell carcinomas reported by Macomber *et al.* (1959) were recurrences after previous treatment.

The 5–10 mm margin recommended by Beirne & Beirne (1959) was based on their experience with tumours treated by Mohs chemosurgery. Beirne & Beirne recorded the number of chemosurgical excisions required to reach a cancer free plane after the bulk of tumour was removed by curettage. On the basis of a 1–2 mm thick layer of tissue being removed at each excision, they concluded that 'effective treatment of carcinomas of the skin by any procedure requires removal of a margin of healthy tissue 0·5–1 cm deep in all directions.' Such sweeping conclusions are not warranted since these authors were treating a very highly selected group of carcinomas. Unfortunately no data as to tumour size, type of tumour, or previous therapy were given by Beirne & Beirne, but it is reasonable to conclude that the majority of their tumours represented 'problem' cases in terms of infiltration, recurrence, location, and size, since chemosurgery is not a primary treatment for the small uncomplicated basal cell carcinoma.

The observation that, in small basal cell carcinomas, a 2 mm margin of normal tissue ensures surgical margins free of tumour in over 95% of patients has implications in the controversy between excision versus curettage and electrodesiccation as the treatment of choice for early basal cell carcinoma. Advocates of the curettage technique usually cite two advantages: (1) relatively little normal tissue is destroyed, and (2) the curetting procedure aids in delineating the tumour by the characteristic 'feel' of the neoplasm. How much 'normal' tissue is destroyed by curettage and electrodesiccation? Popkin (1968) advocates destruction of a 3–5 mm margin of normal tissue. Tromovitch (1965) recommends two separate electrocoagulations each time involving a 2–4 mm band of tissue. Both these authors suggest treatment zones significantly greater than 2 mm, although it is clear that excision differs from electrocoagulation destruction in that electrocoagulation about the tumour is not routinely carried into the subcutaneous fat.

The 'characteristic' feel of the basal cell carcinoma when curetted is repeatedly stressed as being decisive in delineating the extent of the tumour. This feel is often of help in diagnosing a basal cell carcinoma clinically suspected of being a keratosis when on curettage the curette 'falls' into the soft tumour mass. Depth perception by curettage yields no advantage *vis-à-vis* excision since the deep border of a proper surgical excision is in the plane of the subcutaneous fat. Early basal cell carcinomas rarely penetrate to this depth, and when they do, the curette is of no help in delineating such penetration since the normal subcutaneous fat is just as soft as the tumour.

As to the advantage of curettage in delineating lateral margins, the curette can hardly improve on the accuracy of careful visual assessment as documented by this study. It appears that the superiority claimed for the curette assessment of lateral margins is dermatological dogma rather than established fact.

When dealing with infiltrating (sclerosing) tumours, visual assessment of margins is often difficult and sometimes inadequate. Unfortunately, in infiltrating tumours, the curette is usually incapable of delineating the extent of the lesion since the infiltrating epitheliomas characteristically lack the mushy feel essential for treatment by curettage. In summary, it appears that lateral margins can be detected as well visually as by curettage in nodular and superficial basal cell carcinomas; in the infiltrating type of tumour both visual assessment and curettage are often inadequate.

Successful treatment of basal cell carcinoma using a 2 mm margin requires not only accuracy in delineating the tumour, but also attention to surgical technique with clean vertical incisions into the subcutaneous fat. It is important to avoid timid surgery with 'nibbling' at the excision, as well as any departure from a vertical incision. Accurate assessment of visual margins demands excellent light and is greatly facilitated by using a binocular magnifier of moderate (2·5 ×) power. Pressure on the tumour area, and stretching the surrounding skin with the fingers often brings into sharp relief an otherwise obscure tumour border. I have found pre-operative marking, and the need to commit myself to a definite margin, very helpful and have continued routinely to mark the exact tumour margins as a preliminary step to excision. Of course this must be done before local anaesthesia distorts landmarks.

This study documents the accuracy of visual assessment of margins in early basal cell carcinoma, and implies that the traditionally recommended 5–10 mm margins of normal tissue are unnecessarily wide. The author considers conservative surgical excision the treatment of choice for the early basal cell carcinoma. The amount of tissue destroyed need be no greater than with the curettage and desiccation, yet healing is faster and the cosmetic results superior. Furthermore, the pathologist receives a single, intact specimen and is able to examine the tissue with step level sections for completeness of excision. It is hoped that emphasis on the adequacy of a 2 mm surgical margin will stimulate increased use of surgical excision as a primary treatment for early basal cell carcinoma.

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Fungal infection of skin and nails by *Hendersonula toruloidea*

C.K.CAMPBELL, AZIZ KURWA, A-H.M.ABDEL-AZIZ*
AND C.HODGSON

MRC Unit on the Experimental Pathology of the Skin, The Medical School,
The University, Birmingham 15 and The Skin Hospital, Birmingham 15

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SUMMARY

Ten cases of skin and nail infection by *Hendersonula toruloidea* are described. All the patients were from tropical countries, though some had been in Britain for many years. Infections were confined to the hands and feet, which showed scaling skin and dystrophic nails. Four of the patients presented with these symptoms, the remaining six having presented with other skin conditions. The presence of brown-walled hyphae in nail tissue and the repeated isolation of *H. toruloidea* in pure culture are taken as evidence that this species was infecting the tissues. In each of two patients there was evidence of concurrent infection by a ringworm fungus in one of the sites examined.

Gentles & Evans (1970) were the first to present evidence of the possible pathogenicity to man of the plant parasite *Hendersonula toruloidea*. This species was first described in full by Natrass (1933) as a cause of die-back of stone fruit trees in Egypt. It has since been recognized as a cause of branch wilt, canker and die-back diseases of a very wide range of trees, many of them of economic importance (Calavan & Wallace, 1954; Paxton & Wilson, 1965; Punithalingam & Waterston, 1970) and as a cause of fruit rot of bananas (Meredith, 1963). Its geographical range as a plant pathogen is pan-tropical, involving Central America, Africa and southern Asia.

Gentles & Evans (1970) described infection of nails and skin of feet of eight males, seven of whom were immigrants to Britain from tropical countries. All showed a clinical picture resembling tinea pedis, but *H. toruloidea* was repeatedly cultured. In only two cases were dermatophytes isolated.

The present paper reports ten further cases of hand and feet infection in which *H. toruloidea* was implicated, with particular emphasis on their clinical presentation. One of the cases (Case 1) was reported by Kurwa & Campbell (1972).

MATERIALS AND METHODS

The patients were referred to dermatological departments in the Birmingham area. Samples of skin and nail were examined by one of us (C.K.C.) as part of a general survey to ascertain the pattern of arrival and spread of the non-indigenous mycoses in the West Midlands.

Laboratory methods involved the direct microscopical examination of KOH squash preparations of samples, and culture on two media: (a) SAC medium, Sabouraud glucose agar (Oxoid), incorporating 0·05 mg/ml chloramphenicol and 0·5 mg/ml cycloheximide; (b) MC medium, malt extract agar (Oxoid), with 0·5 mg/ml chloramphenicol. Incubation was at 27°C for at least 2 weeks.

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* Present address: Department of Dermatology and Venereology, Al-Azhar University, Cairo, Egypt.

The assays of sensitivity of *H. toruloidea* to griseofulvin (Glaxo Laboratories Ltd) were carried out using a range of 3 ml aliquots of two-fold dilutions of the antibiotic in glucose peptone broth. The range covered was from 100 μg/ml to 0·18 μg/ml. These were each inoculated with 0·1 ml of an aqueous suspension of arthospores, obtained by flooding a malt agar slope culture, and incubated at 27°C for 4 weeks, after which the presence or absence of growth was recorded.

CASE HISTORIES

Case 1

A 52-year-old Jamaican male, resident in the Britain for 28 years, presented with chronic paronychia and nail plate dystrophy of most fingers. He had noticed abnormal toe-nails since his early childhood. On examination, the finger-nails showed disintegration and separation of the nail plate at the distal end. The nails of the right great, 2nd and 4th toes were thickened and partly destroyed (Fig. 1), and the left great toe-nail showed sub-ungual hyperkeratosis. All the affected nails exhibited a brown discoloration along their sides. There was also scaling of both soles, which was most marked in the creases. The affected areas did not fluoresce with Wood's light.

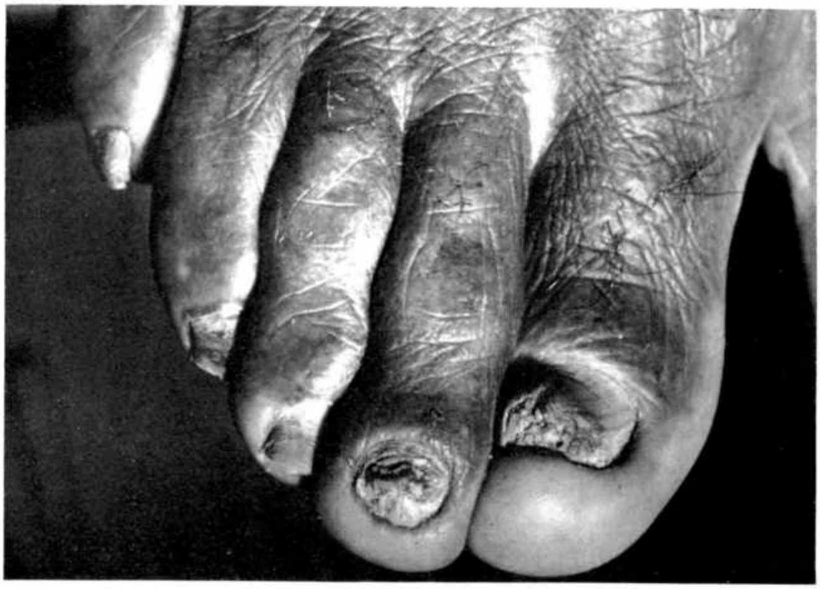

FIGURE 1. Case 1; toes of right foot, showing nail dystrophy and hyperkeratosis.

Case 2

A 53-year-old Jamaican male, resident in Britain for 20 years, presented with urticaria of 4 years' duration. There was also scaling of the soles, toe clefts and the right palm. The toe-nails of both feet were dystrophic. Chronic paronychia of the right little and ring fingers was present.

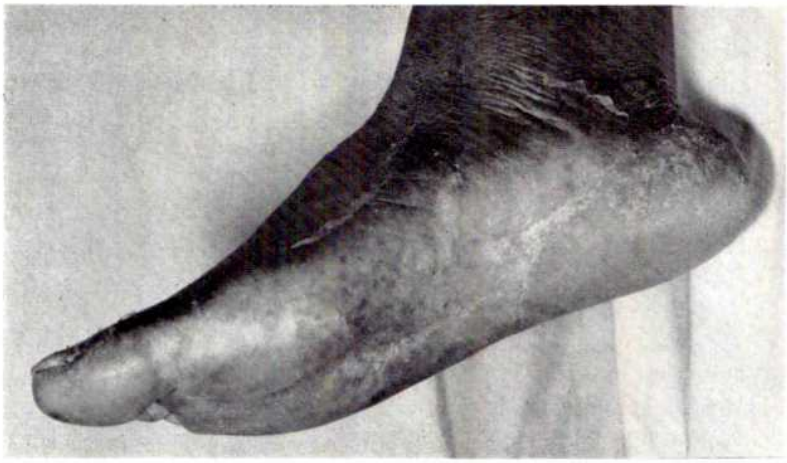

FIGURE 2. Case 3; right foot, showing scaling skin.

Case 3

A 46-year-old Jamaican male, resident in Britain for 16 years, presented with cheiropompholyx of 1 week's duration. On examination there was asymptomatic scaling of the feet and toe clefts (Fig. 2). The toe-nails were yellow, thickened and showed sub-ungual hyperkeratosis. There was no fluorescence of the affected areas under Wood's light.

Case 4

A 56-year-old Jamaican male, resident in Britain for 11 years, had diabetes mellitus of at least 6 years' duration. He presented with chronic paronychia of the left index finger (Fig. 3), the nail of which was thickened, brownish and partially destroyed. The left great, 3rd, 4th and 5th, and the right great, 4th and 5th toe-nails were thickened and dystrophic. These areas did not fluoresce under Wood's light.

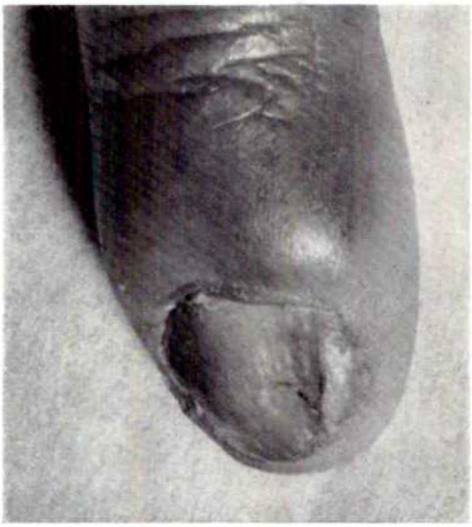

FIGURE 3. Case 4; left index finger, with nail changes and paronychia.

Case 5

A 50-year-old Jamaican female, resident in Britain for 8 years, presented with blackened, distorted toe-nails. On examination there was onychogryphosis and marked sub-ungual hyperkeratosis together with scaling of the 4th toe clefts.

Case 6

A 37-year-old Pakistani male, resident in Britain for 2 years, presented with seborrhoeic dermatitis and folliculitis of 2 years' duration, and on examination he also showed interdigital scaling of the feet.

Case 7

A 34-year-old Pakistani male, resident in Britain for 3 years, presented with gross destruction and yellow discoloration of all nails of both fingers and toes (Fig. 4) and scaling of the toe clefts and soles.

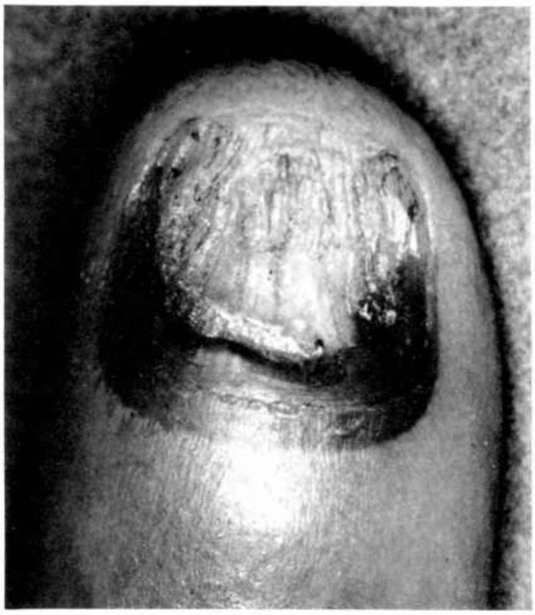

FIGURE 4. Case 7; left index finger, showing nail erosion and discoloration.

Case 8

A 35-year-old Pakistani male, resident in Britain for 9 years, except for a return to Pakistan between 1969 and 1971, presented with a macular melanoderma of trunk and limbs of 3 months' duration, the cause of which has not been determined. He was unaware of scaling of his soles, toes and, to a lesser extent, palms. The toe-nails were thickened, yellowish and partially destroyed.

Case 9

A 51-year-old Indian male, resident in Britain for 5 years, presented with chronic eczematous dermatitis of the limbs of 9 months' duration. The nails of the right thumb, right index finger, left ring finger and of the great toes were yellowish, thickened and showed sub-ungual hyperkeratosis. There was also scaling of the right palm. The hands and feet did not fluoresce under Wood's light.

Case 10

A 38-year-old Pakistani male, resident in Britain for 12 years, presented with a painful neurotrophic ulcer with callosity and hyperkeratosis on the sole of the left foot, the dorsum of which was hyperpigmented. There was a loss of sensation of the 3rd, 4th and 5th toes, which were deformed with atrophy of the subcutaneous tissues. The sides of the feet were hyperkeratotic and the toe-nails were-thickened.

MYCOLOGY

A summary of the mycological findings in the various sites of the patients is given in Table 1. Many of the samples contained hyphae which could not be distinguished from those of dermatophytes in KOH squash preparations, but some features of difference were noticed. In two samples of finger-nail, from Cases 1 and 4 respectively, some of the hyphae were brown-walled (Fig. 5), resembling the older hyphae of *H. toruloidea* in culture. In other samples, especially from the skin, the hyphae were very contorted, with considerable difference in width along any one length. Arthrospores in both nail and skin were 'long-oblong', unlike the short rounded spores usually found in Trichophyton species.

TABLE 1. Results of microscopical examination and culture from various sites

			Cultural results	
Case	Site	Direct examination	Malt agar + chloramphenicol	Sabouraud's agar + chloramphenicol + cycloheximide
1	Toe-nail	+ (3)*	*H. toruloidea* (3)	—
	Foot	+	*H. toruloidea*	—
2	Finger-nail	+ (3)	*H. toruloidea* (2)	—
	Toe-nail	+ (2)	*H. toruloidea*	—
	Foot	+	*H. toruloidea*	*T. mentagrophytes*
3	Toe cleft	+ (2)	*H. toruloidea*	—
	Sole	+ (2)	*H. toruloidea*	—
4	Toe-nail	+ (2)	*H. toruloidea*	—
	Finger-nail	+ (2)	*H. toruloidea* (2)	—
5	Toe-nail	+	*H. toruloidea*	—
	Toe cleft	+	*H. toruloidea*	—
6	Foot	+	*H. toruloidea*	—
7	Toe-nail	+ (3)	*H. toruloidea* (3)	—
	Finger-nail	+	—	—
	Sole	+	—	—
	Toe cleft	+	—	—
8	Toe-nail	+ (4)	*H. toruloidea*	—
	Toe cleft	+ (5)	*H. toruloidea* (5)	*T. rubrum* (3)
	Sole	+ (4)	*H. toruloidea* (3)	—
	Palm	+ (3)	*H. toruloidea* (4)	—
9	Toe-nail	+ (2)	*H. toruloidea*	—
	Foot	+	*H. toruloidea*	—
	Palm	+	*H. toruloidea*	—
10	Foot	+	*H. toruloidea*	—

* Figures in brackets refer to the number of separate occasions on which samples have given the same result.

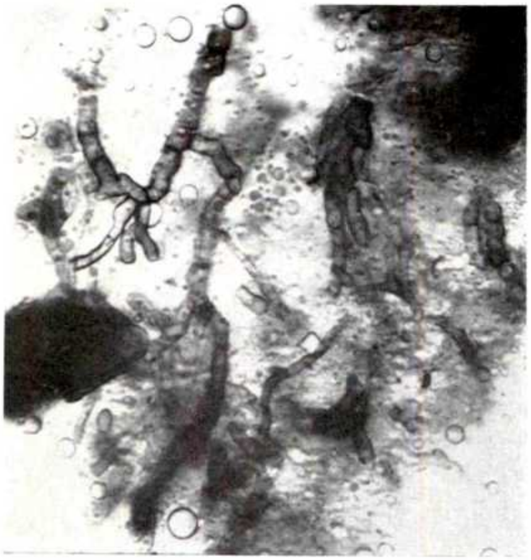

FIGURE 5. Case 1; KOH squash preparation of nail tissue, showing brown-walled hyphae.

Table 1 shows that in many instances there have been repeated isolation of *H. toruloidea* in the absence of any dermatophyte and that it grew only on the cycloheximide-free MC agar. Mixed infections were demonstrated in the foot sample from Case 2 (*H. toruloidea* and *Trichophyton mentagrophytes*) and in the toe cleft of Case 8 (*H. toruloidea* and *Trichophyton rubrum*). The skin samples from the areas of melanoderma of Case 8 (chest) and the eczema of Case 9 (leg) showed no evidence of fungal infection.

The isolates of *H. toruloidea* obtained showed considerable variation in appearance, and a detailed study of these will be published separately. The strains were morphologically stable on repeated subculture, and similar strains were always obtained on further isolation from any one site.

Most strains exhibited fast growth, filling an 85 mm Petri dish in 2–3 days. The aerial growth was high, dense, initially white, later mouse grey, whilst the reverse was brownish grey to black.

Microscopically there was an abundance of arthrospores formed from the fragmentation of hyphae. These spores were 2·5–10(5) × 2·5–5(4) μm, long-cylindrical to barrel-shaped, colourless to olive brown. Strains from Cases 5, 6, 7 and the palms and soles of Case 8 were much slower growing with less aerial mycelium and a lesser tendency to arthrospore production.

THERAPY

Various antifungal drugs have been tried on some of the patients, and whilst it is too early to draw conclusions as to their effectiveness, initial impressions are that griseofulvin is not beneficial. *In vitro* estimation of the sensitivities to this antibiotic of *H. toruloidea* strains isolated from Cases 1, 2, 8 and 9 showed no inhibition of growth at the highest concentration tested (100 μg/ml). This is in agreement with the findings of Gentles & Evans (1970).

DISCUSSION

The cases presented above lend support to the view proposed by Gentlgs & Evans (1970) that *H.*

toruloidea is capable of invading skin and nail tissue and giving a clinical appearance which resembles dermatophytosis of the hands and feet. The most convincing evidence, apart from the repeated isolation of this species from the same site on several patients, is that brown-walled hyphae have been found in some of the samples. Such hyphae could not have belonged to a true dermatophyte. More knowledge is needed before the term pathogenic can be strictly applied to this fungus, however, since its part in producing the host response, and its status as a primary invader are not fully established. The existence of multiple fungus infections is well recognized (Partridge, 1955) and it seems reasonable to postulate double infections in the two instances (Cases 2 and 8) in which dermatophytes were isolated together with *H. toruloidea*.

The genus *Hendersonula* is a member of the Sphaeropsidales, a large group of asexual fungi which produce spores within flask-shaped structures known as pycnidia. Such pycnidia have so far been observed in strains from Cases 1, 2, 3, 4 and 8. The identification of the remaining isolates has been based upon their production of brown arthrospores.

As this fungus is not a close relative of the ringworm fungi, it is not surprising that it differs from them in physiological respects, such as its susceptibility to cycloheximide and its tolerance of griseofulvin. Its inability to grow in the presence of cycloheximide probably accounts for its remaining unnoticed by medical mycologists until recently, since most laboratories use this compound for the isolation of ringworm fungi.

Seven of the ten cases described here showed nail involvement, compared with only two of the eight reported by Gentles & Evans (1970). A single case found by Dr R.R.Davies (personal communication) in 1964, and two others seen by Dr M.P.English (personal communication), all involved nails. It is difficult to select any one clinical feature of the nails which may be pathognomonic for this infection. As in other types of onychomycosis some of the nails on the affected hand or foot remained normal, although other nails had apparently been infected for many years.

The nails in the present cases were generally dystrophic and exhibited yellowish to brownish-black discoloration. These changes appeared to begin at the nail edges, spreading to involve the whole nail-plate. The degree of dystrophy, separation of nail and subungual hyperkeratosis were variable.

H. toruloidea is apparently unusual in its ability to invade the skin of hands and feet, since other non-dermatophyte pathogens are typically restricted to infections of nail tissue (Walshe & English, 1966; English, 1968; Zaias *et al.*, 1969). As in ringworm infections of feet and hands, the factors restricting the fungus to these sites are unknown.

The skin infection did not cause physical discomfort to the patients. In only four cases was the *H. toruloidea* infection the primary reason for attending the skin clinics, and some of the patients did not realize their feet and hands were abnormal.

At present there is no reason to suppose that the *Hendersonula* infections were connected with the presence of the other conditions (urticaria, pompholyx, eczematous dermatitis, melanoderma and diabetes) found in the patients. Chronic paronychia was present in three patients (Cases 1, 2 and 4) and in two it was the presenting symptom, although there is no proof that it was the direct result of *H. toruloidea* infection.

The patients had been resident in Britain for between 3 and 28 years, and because of the geographical distribution of the fungus it is assumed that the infections were contracted in the patients' countries of origin and had remained undetected for many years. The origins of the patients suggest that there is a distinct probability that the infection occurs among the populations of Jamaica and the Indian sub-continent, and perhaps wherever the fungus is an indigenous plant pathogen.

The possibility that the infection is transmissible by means of infected skin and nail debris needs investigation. Work by one of us (C.K.C.) has shown that infected skin scales kept dry at roots temperature for 6 months still contained viable fungus, so that the potential for the spread of infection appears to exist.

ACKNOWLEDGMENTS

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Pharmacology and Treatment

The placebo response to white soft paraffin/propylene glycol in the skin blanching test

R.WOODFORD AND B.W.BARRY

School of Pharmacy, Portsmouth Polytechnic, Park Road, Portsmouth

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SUMMARY

Eight grades of white soft paraffin BP were investigated for their ability to produce pallor of normal skin in a test designed originally for topical corticosteroid activity. All samples produced definite but transient blanching, the extent of which was determined by the grade of white soft paraffin and the test method employed. Pallor was often more pronounced under non-occlusive test conditions and when the sample contained 5% w/w propylene glycol. Blanching may have been due to the presence of a pallor-inducing substance transported into the skin by propylene glycol, rather than to a simple hydration effect.

McKenzie & Stoughton (1962) showed that skin application of corticosteroid solutions produced blanching at the site of application. As a result they devised a simple method to investigate the activity of topical steroids. The mode of action of corticosteroids in inducing pallor of normal skin remains obscure, but is possibly mainly due to decongestion of the capillaries (Thune, 1971). A relationship has been demonstrated between the ability of topically applied steroids to produce blanching and to alleviate inflammation in therapeutic use (Stoughton, 1969; Place, Velazquez & Burdick, 1970). Modifications of the blanching test have proved valuable in investigation of the effect of formulation variables on cutaneous response to steroid application (Barrett *et al.*, 1965; Pepler, Woodford & Morrison, 1971; Coldman, Lockerbie & Laws, 1971a; Ostrenga *et al.*, 1971).

It is often assumed that bases alone produce no blanching, but whether topical vehicles produce pallor or not may depend on the nature of the formulation and on the test conditions employed. Occlusive testing produced blanching in some cases (Burdick, Poulsen & Place, 1970), but not in others (Reid & Brookes, 1968), while pallor occurred under non-occlusive conditions (Coldman & Lockerbie, 1971). The effect of test design and of propylene glycol on blanching produced by different grades of white soft paraffin BP were investigated because such combinations are often used in the development and presentation of topical steroid formulations.

MATERIALS AND METHODS

White soft paraffin bases

Eight samples of white soft paraffin BP were obtained from two suppliers. Grades 1–5 were purchased from the first company (Dalton & Co. Ltd, Belper, Derbyshire) at the same time, whereas grades 6–8 were obtained from the second supplier (J. M. Loveridge Ltd, Southampton, Hants.) over a 2-year period. Bases containing propylene glycol BP were prepared by incorporating the glycol using the tile method.

Volunteers

Ten volunteers were selected without reference to their sex or steroid sensitivity. None had received topical application of corticosteroids for at least 6 months prior to the investigation.

Methods

The blanching test employed was a modification of that described by Christie & Moore-Robinson (1970) and incorporated a double blind technique. 10 mg quantities of eight grades of white soft paraffin BP were applied to the washed flexor surfaces of both forearms. The application sites consisted of 7 × 7 mm areas punched out from Blenderm polyethylene tape (3M Medical Products, London) to which the bases were applied by reference to previously-prepared randomization sheets. The sites were occluded with type S 12 μm Melinex polyester film (ICI Plastics Division, Welwyn Garden City) for 6 h (method a). The areas were washed with soap and water at skin temperature, dried, and the degree of pallor estimated 10 min later using a 0–3 scale: 0, normal skin; 1, slight pallor of indistinct outline; 2, pallor with at least two corners outlined; 3, even pallor with a clear outline of the square.

The experiment was repeated (method b) replacing the Melinex film by a perforated metal screen, and (method c) as method (b) but removing the Blenderm tape before placing the screen in position.

All experiments were repeated with samples of white soft paraffin containing 5% w/w propylene glycol. In addition, white soft paraffin grades 3 and 5 containing varying amounts of propylene glycol were tested by method (a) only.

All estimations of pallor were performed using the same lighting conditions and without reference to the application charts. In every case the readings were taken hourly until blanching disappeared.

The effect of using a combination of white soft paraffin and Melinex film on skin temperature at the application site was determined as follows. A bead-type thermistor (TH-B12, Radiospares Ltd, London) was embedded in white soft paraffin applied by method (a), the Blenderm tape and Melinex film holding the thermistor rigidly in place. A second thermistor was embedded alongside the occluded site in a similar manner except that only a small strip of Blenderm tape was used to keep the thermistor in position, and the Melinex film was omitted. Thus, the temperature measurement sites were analogous to the application areas of methods (a) and (c). The thermistors were connected in turn across the terminals of an Autobalance Universal Bridge (type B641, Wayne Kerr, Chessington) and their resistances measured over a 6 h period. The readings were converted to degrees centigrade using previously-prepared calibration curves of resistance against temperature obtained with a NPL 0–40°C thermometer.

RESULTS

The amount of skin pallor produced by different grades of white soft paraffin alone and white soft paraffin containing 5% propylene glycol is shown in Figs. 1–3 as a percentage of the total possible score (note ordinate scale expansion). Preliminary experiments showed that 10 min was the optimum time for reading the initial (6 h) responses. At shorter intervals between drying and reading the estimates of pallor were less reliable, probably due to mechanical friction of the blanched area during the drying process. Because the skin pallor was fleeting (all blanched areas had disappeared within 3 h) readings obtained subsequent to 10 min following drying were not regarded as being initial responses.

There was no apparent relationship between the ability of a white soft paraffin sample to induce

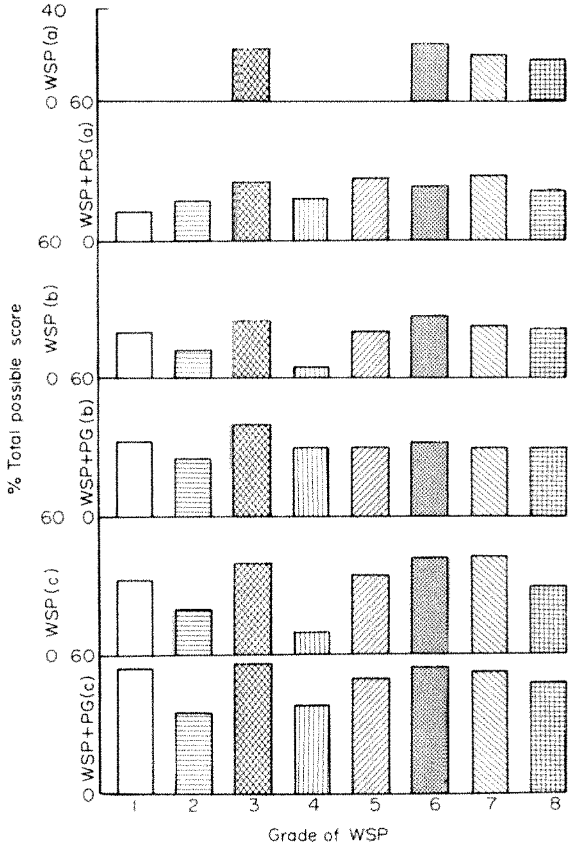

FIGURE 1. Effect of white soft paraffin grade and application method on skin blanching, 6 h response. WSP, white soft paraffin; PG, 5% w/w propylene glycol; (a) (b) (c), application methods.

blanching and its rheological characteristics (Barry & Grace, 1971). Similarly, there was no obvious correlation between the presence of residual white soft paraffin at the application site after 6 h and the amount of pallor produced.

The 6 h responses were subjected to an analysis of variance, using a three-factor (3 × 2 × 8) factorial design as a fixed-effect model (Winer, 1962; Goldstein, 1964). The results of that analysis show that, for the experiment as a whole, there were statistically highly significant differences between the methods of testing (F = 127·41**), the presence and absence of propylene glycol (F = 96·76**) and the grade of white soft paraffin employed (F = 14·78**). However, '*t*' tests showed no significant difference between samples 1 and 5; 2 and 4; and 3, 6, 7 and 8 for any given test method in the presence or absence of propylene glycol. Sample 3 is the parent grade of series 1–5, from which the others are derived, and it is possible that samples 6, 7 and 8 are different batches of grade 3. The amount of pallor visible 7 and 8 h after application essentially followed the same rank order of bases as the 6 h results.

Re-occlusion of the application sites with Melinex film after 24 h (Vickers, 1963) failed to elicit blanching, indicating that no significant reservoir of pallor-inducing substance was produced in the stratum corneum. Neither propylene glycol (1 μl) nor water produced blanching when tested by method (a), (b) or (c). One week later white soft paraffin samples, alone and containing 5% propylene glycol, were re-applied by method (a) to five volunteers. The results did not differ significantly from

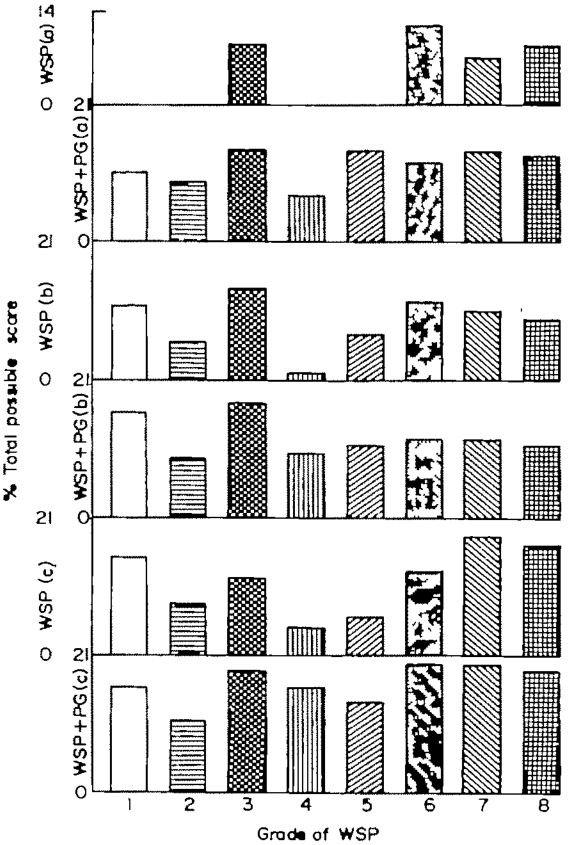

FIGURE 2. Effect of white soft paraffin grade and application method on skin blanching, 7 h response. WSP, white soft paraffin; PG, 5% w/w propylene glycol; (a) (b) (c), application methods.

those obtained in the first experiment, suggesting that any variation in blanching in the same individual at different times (McKenzie, 1966) was insignificant.

Of the white soft paraffin samples investigated, grade 3 showed the minimum difference under occlusion between the presence and absence of 5% propylene glycol, while grade 5 exhibited the maximum difference in skin pallor in the presence and absence of 5% propylene glycol (Fig. 1). The effect of various percentages of propylene glycol on skin blanching produced by white soft paraffin grades 3 and 5 under occlusion is shown in Fig. 4. This figure indicates that where the presence of 5% propylene glycol had minimal effect (white soft paraffin grade 3) the degree of blanching was essentially independent of the amount of propylene glycol incorporated into the base. On the other hand, where the difference between the presence and absence of propylene glycol was maximal (white soft paraffin grade 5) the amount of pallor produced generally increased with propylene glycol content to a maximum at 7% w/w and then remained constant. The difference between the two grades tended to diminish at the 8 h response.

Use of the thermistors showed that covering white soft paraffin with Melinex film (method a) resulted in a temperature increase at the skin surface of approximately 0·7°C over the site not occluded with Melinex (method c). This temperature difference was attained within 90 min and remained approximately constant for the duration of the experiment. The temperature of normal skin as well as

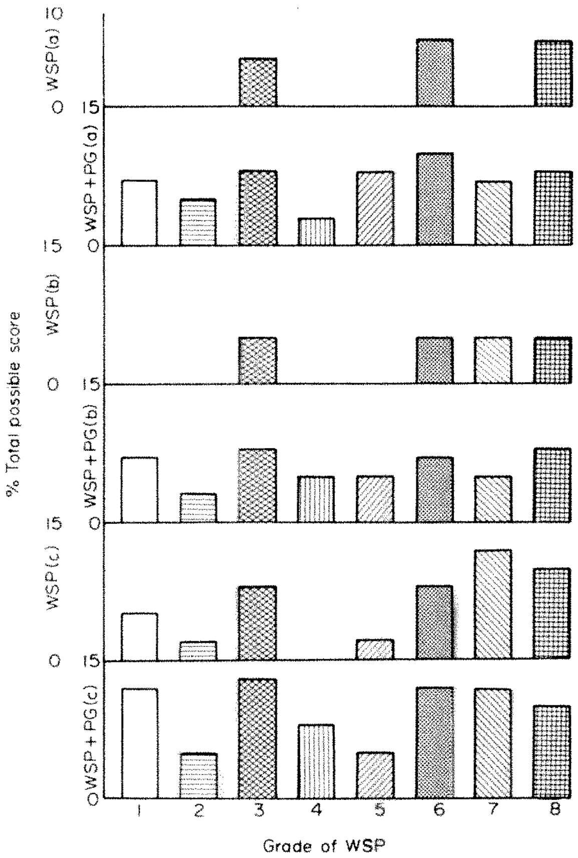

FIGURE 3. Effect of white soft paraffin grade and application method on skin blanching, 8 h response. WSP, white soft paraffin; PG, 5% w/w propylene glycol; (a) (b) (c), application methods.

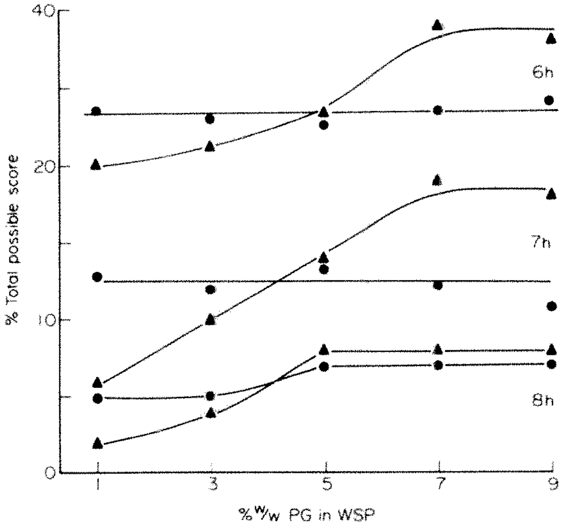

FIGURE 4. Effect of propylene glycol on skin blanching produced by two grades of white soft paraffin. WSP, white soft paraffin; PG, propylene glycol; ●—●, white soft paraffin grade 3; ▲—▲, white soft paraffin grade 5; test method (a) employed.

sites occluded by method (a) or method (c) varied with the time of day, the maximum value being attained in the early afternoon, as found by Shahidullah *et al.* (1969).

DISCUSSION

The results show that white soft paraffin BP produced a definite but transient blanching in a topical test for corticosteroid activity. The amount of pallor produced was determined by the grade of white soft paraffin, the method of testing and the presence of propylene glycol.

Coldman *et al.* (1971a), using method (c) as described in this paper, found that white soft paraffin BP produced an initial blanching response of 50·5% total possible score. This was reduced by half 16 h later but even 48 h after application the amount of pallor was estimated as high as 20% maximum possible value. Similarly high values were obtained with a series of white soft paraffin bases containing 5% propylene glycol, the magnitude of the responses depending on the composition of the white soft paraffin (Coldman & Lockerbie, 1971), and with various placebo ointments and creams (Coldman *et al.*, 1971b).

In the present work the initial readings of skin pallor were comparable to those obtained by Coldman *et al.* (1971a, b) and Coldman & Lockerbie (1971). However, while Coldman was able to detect blanching for at least 1·5–2 days following application, in the present work all responses had disappeared within 3 h. The persistence of pallor noted by Coldman may have been caused by a residual reservoir effect from earlier tests using corticosteroid formulations (although this was discounted) or by possible contamination of materials by traces of steroid in the test environment.

Higher responses were obtained under non-occlusive test conditions than when the application sites were covered with Melinex film. It is possible that some vasodilation produced under the warmer occlusive conditions may have contributed towards the reduced skin pallor, although this effect would be minimal because of the small temperature differential (0·7°C) observed between the two techniques. However, the presence of Blenderm tape produced occlusion and occasional erythema in the area surrounding the application site; these may have contributed towards an increased microcirculation in that region leading to more rapid removal of pallor-inducing substance. In some cases removal of the Blenderm tape left a region that was rather paler than normal skin. Assessment of pallor against that background may have resulted in somewhat lower scores being allocated. The effect of a larger response resulting from non-occluded application of white soft paraffin bases is in contradistinction to the greater degree of pallor produced at occluded sites of corticosteroid formulations (Burdick, 1972a, b; Ostrenga *et al.*, 1971).

The addition of propylene glycol to white soft paraffin resulted in increased pallor, and the greater the effect of propylene glycol under occlusion the more pronounced was the effect of increasing concentration of propylene glycol. These results may be due to different amounts of an unknown 'blanching substance' present in the white soft paraffin samples which were transported into the skin by propylene glycol. If that was the case, where the amount of 'blanching substance' was small, 1% propylene glycol was sufficient to produce the effect (white soft paraffin grade 3) but larger quantities of substance required higher percentages of propylene glycol to elicit a maximum response (grade 5). Extraction of white soft paraffin grades 3 and 5 by propylene glycol resulted in turbid glycol solutions, but the latter failed to produce pallor on open or occluded application sites.

Berube, Messinger & Burdick (1971) prepared five white soft paraffin bases complying with the USP monograph for white petrolatum which differed in their occlusive properties. Coldman & Lockerbie (1971) suggested that the placebo response to white soft paraffin may be due to a whitening of the skin due to increased hydration at the application site. If different grades of white soft paraffin varied in occlusivity they would be expected to produce different degrees of hydration and resultant skin pallor. In the present work both occlusion of normal skin and occlusion of varying amounts of water with Melinex film for 6 h failed to induce blanching. This indicates that pallor induced by white soft paraffin may not be due to a simple occlusion effect.

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A comparison of the effect of various topically active steroids on the clinical and histological features of psoriasis

P.G.GOODWIN, SUSAN HAMILTON AND LIONEL FRY

Department of Dermatology, St Mary's Hospital, London, W.2

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SUMMARY

The effect of betamethasone 17-valerate, fluclorolone acetonide, fluocinolone acetonide and formocortal on the clinical and histological features and mitotic counts of psoriatic epidermis have been compared.

In five of the six patients studied the preparations were used under polythene occlusive dressings. Biopsies were carried out before treatment and then at varying intervals for a week. All the four steroid preparations appeared effectively to reverse the psoriatic lesion.

Betamethasone 17-valerate and fluocinolone acetonide were found to give similar and slightly superior results to fluclorolone acetonide and formocortal on the criteria used in this study.

It would appear that at the present time more potent topical steroids would not be of any advantage in the treatment of psoriasis. Drugs with a more selective action on the psoriatic process and without the steroid effect on collagen are required.

Histological observations of psoriatic epidermis treated with topical corticosteroid preparations show that the earliest change to take place in the clearing of psoriasis is the reformation of a granular layer. This is closely followed by a fall in the mitotic count in the basal layer (Fry & McMinn, 1968). The mode of action of topical corticosteroid drugs in psoriasis is at present unknown. However, it is well known that their effectiveness in clearing psoriasis can be increased by polythene occlusive dressings, and Fry, Almeyda & McMinn (1970) showed that polythene occlusion alone caused significant reformation of the granular layer and a fall in the mean mitotic counts of psoriatic epidermis compared with a control group.

There are now many potent steroid preparations used in psoriasis and it is possible that the current screening procedures used to determine the potency of the steroid do not necessarily select the action by which it has a beneficial effect on the psoriatic process. We have, therefore, compared the effects of a number of these steroid preparations on the histological and cytological features of psoriasis to determine if these correlate with the known potency of these preparations as determined by the standard screening tests, i.e. vasoconstriction, thymus atrophy, granuloma reduction and clinical results in the treatment of psoriasis.

PATIENTS AND METHODS

Patients

Six patients with symmetrical psoriasis involving the forearms were studied. None had received treatment for at least 2 weeks before the study. Three males and three females were studied and their ages ranged from 29 to 63 years (mean 49).

Steroid preparations

The corticosteroids studied were betamethasone 17-valerate (0·1%), fluocinolone acetonide (0·025%), formocortal (0·025%), and fluclorolone acetonide (0·025%). Three groups were studied; in each group betamethasone 17-valerate was compared with the other three corticosteroids. The groupings were selected on the basis of previously reported clinical results (obtained in the treatment of psoriasis).

Group 1. Formocortal 0·025% has been shown to be inferior to betamethasone 17-valerate 0·1% (Marks & Garrett, 1972).

Group 2. Fluocinolone acetonide 0·025% which, after 8 years of clinical use, appears to be equally effective as betamethasone 17-valerate 0·1%, although the initial report of Williams *et al.* (1964) found betamethasone 17-valerate to be superior.

TABLE 1. Betamethasone 17-Valerate vs formocortal

Patient no.		Pre-treatment		Duration of treatment							
				24 h		48 h		96 h		7 days	
		L	R	L	R	L	R	L	R	L	R
1. (right: formocortal, left: betamethasone 17-valerate)				L = R		L>R		L>R		L>R	
	C	4	4	3	3	2	2	1	2	1	2
	G	0		1	1	2	1	3	2	3	3
	M	74		74	80	29	28	18	20	2	2
5. (right: betamethasone 17-valerate, left: formocortal)				L = R		L<R		L<R		L<R	
	C	4	4	3	3	3	2	3	1	3	1
	G	0		0	1	1	1	1	2	1	2
	M	64		76	60	44	40	40	28	36	18

C, clinical score; G, granular layer score; M, mitotic count.

Group 3. Fluclorolone acetonide 0·025% which has been shown to be superior to betamethasone 17-valerate (Harman *et al.*, 1972).

In each group there were two patients. Corticosteroids in ointment were packed in identical tubes. Each patient was given two tubes labelled left and right. The ointments were applied daily under polythene occlusive dressings in five patients and twice daily without occlusive dressings in one patient (5).

The patients were studied for 1 week, as when topical steroid preparations are used in psoriasis, the most significant clinical and histological changes occur during this period (Fry & McMinn, 1968). The patients were seen at 24, 48, 96 h and 1 week after commencement of treatment.

Clinical assessment

The clinical severity of the skin lesions was assessed as a numerical score: 4, scaly palpable lesion; 3, minimal scaling but easily palpable; 2, lesion just palpable; 1, erythema; 0, clear.

Histological assessment

Punch biopsies (4 mm) were carried out using 2% lignocaine as the local anaesthetic. The biopsies were taken from both arms, before treatment and then at 24, 48, 96 h and at 1 week. The specimens were fixed in formol-saline, embedded in wax and stained with haematoxylin and eosin.

TABLE 2. Betamethasone 17-valerate vs fluclorolone acetonide

Patient no.		Pre-treatment		Duration of treatment							
				24 h		48 h		96 h		7 days	
		L	R	L	R	L	R	L	R	L	R
2. (right: fluclorolone acetonide, left: betamethasone 17-valerate)				L = R		L>R		L>R		L>R	
	C	4	4	3	3	2	3	1	3	1	3
	G	0		1	1	2	1	2	2	3	2
	M	85		38	46	26	26	12	20	10	23
4. (right: fluclorolone acetonide, left: betamethasone 17-valerate)				L = R		L<R		L<R		L = R	
	C	3	3	2	2	2	1	2	1	0	0
	G	1		1	1	2	2	2	2	3	3
	M	52		36	42	20	24	20	12	4	8

C, clinical score; G, granular layer score; M, mitotic count.

TABLE 3. Betamethasone 17-valerate vs fluocinolone acetonide

Patient no.		Pre-treatment		Duration of treatment							
				24 h		48 h		96 h		7 days	
		L	R	L	R	L	R	L	R	L	R
3. (right: Fluocinolone acetonide, left: betamethasone 17-valerate)				L = R		L = R		L = R		L = R	
	C	4	4	3	3	2	2	2	2	1	1
	G	0		1	1	2	2	2	2	3	3
	M	80		50	72	38	40	19	20	3	4
6. (right: Betamethasone 17-valerate, left: fluocinolone acetonide)				L = R		L = R		L = R		L = R	
	C	4	4	3	3	2	2	2	2	1	1
	G	0		1	1	1	1	2	2	3	3
	M	64		48	58	28	20	4	16	2	2

C, clinical score; G, granular layer score; M, mitotic count.

Granular layer. This was assessed on a scale 0–3: 0, absent granular layer; 1, thin granular layer in small areas; 2, granular layer in most areas; 3, normal granular layer.

Mitotic counts. These were carried out as described by Fry & McMinn (1966).

RESULTS

Clinical

All the patients showed some degree of clinical improvement during the period of study. In comparing betamethasone 17-valerate with formocortal (patients 1 and 5) the clinical improvement was greater with betamethasone 17-valerate in both patients. This was apparent at 96 h in patient 1 in whom polythene dressings were applied, and at 48 h in patient 5 who was not using polythene. Betamethasone 17-valerate also produced a better clinical result than fluclorolone acetonide in both patients (2 and 4); it was noticeable at 48 h. Betamethasone 17-valerate and fluocinolone acetonide (patients 3 and 6) gave similar clinical results.

Histological

Granular layer. This showed improvement in all patients. However, there were differences in the times at which the improvement was first observed. When comparing formocortal with betamethasone 17-valerate the granular layer showed improvement more quickly with betamethasone 17-valerate. In patient 1 in whom polythene occlusion was used the difference between the two steroids was apparent at 96 h and in patient 5 in whom no occlusive dressings were used the difference was apparent at 48 h.

With fluclorolone acetonide the granular layer reformed more quickly with betamethasone 17-valerate in one patient (2) and at the same time with both steroids in patient 4. Rather surprisingly fluclorolone acetonide was the only steroid which failed to reform the granular layer (patient 2) completely with polythene occlusive dressings at one week.

With fluocinolone acetonide and betamethasone 17-valerate there was no difference in the time taken to reform the granular layer.

Mitotic counts. The mitotic counts fell during the course of treatment in all patients. At the end of the 1 week study period, however, the counts were still higher than are found in normal skin, although in patients 1 and 6 they were approaching the figure for normal skin. In patient 5 the relatively high mitotic count could probably be explained by the fact that no polythene dressings were used.

In comparing formocortal with betamethasone 17-valerate there was very little difference in the mitotic counts at all stages of the study, except at 1 week in patient 5, when the fall in the count was greater in the lesion treated with betamethasone 17-valerate. In comparing fluclorolone acetonide with betamethasone 17-valerate there was no significant difference in the fall in the counts although there was a tendency to a greater fall in the lesions treated with betamethasone 17-valerate at the end of the week's study. In comparing betamethasone 17-valerate and fluocinolone acetonide both drugs induced a rapid fall in mitotic counts and with equal speed. At the end of the week the counts were nearly within normal limits.

DISCUSSION

These results have shown that there is a good correlation between the clinical results and reformation of the granular layer when psoriasis is treated with topical corticosteroids under polythene occlusive dressings. Although during treatment there is a fall in the mitotic counts, which follows the reformation of the granular layer, the results are not compatible with the concept that the primary fault in psoriasis is 'uncontrolled mitosis', with subsequent increased transit time of the keratinocytes through the epidermis, and secondary to this a failure of the cell to differentiate. There is now ample evidence

(Fry & McMinn, 1968; Fry, 1968) that in reversing psoriasis to clinically and histologically normal skin the granular layer reforms before a fall in mitotic counts. This concept is now supported by the theory of a feed-back mechanism from the epidermal cell on mitotic activity of the epidermis (Powell, Duell & Vorhees. 1971; Bullough, 1972). Thus, once the epidermal cell differentiates, i.e. forms keratin and therefore a granular layer, mitotic activity falls.

In this study there was little difference in clinical and histological response between the four steroids investigated. Betamethasone 17-valerate and fluocinolone acetonide were found to give identical clinical and histological results, both in their speed of action and end result after 1 week's study. However, betamethasone 17-valerate was found to be slightly superior to both formocortal and fluclorolone acetonide in speed of action in the clinical and histological criteria chosen. In addition, betamethasone 17-valerate also gave a slightly superior result at the end of 1 week's treatment compared to both patients treated with formocortal and to one of the patients treated with fluclorolone acetonide. It is interesting to note that in the patient not using polythene dressings the difference in results between the two steroids was considerably greater. The implication of this finding is that in clinical trials in which the effects of steroids on psoriasis are being compared, the trial should be performed without polythene occlusion. The greater difference is probably obtained because it is known that occlusion *per se* has a beneficial effect on psoriasis (Fry *et al.*, 1970). Our studies also show that, at the present time, the best way of assessing the value of a topical steroid in psoriasis is by clinical trial. There is nothing to suggest that histological studies have anything further to offer in selecting a more potent topical steroid.

This investigation appears also to indicate that current research designed to produce a more potent topical steroid as assessed by vasoconstrictor assay, thymus atrophy or granuloma reduction will not necessarily produce a better drug for the management of psoriasis. Enhancement of the effect of the available steroids can already be achieved by the use of occlusive dressings, and unfortunately this enhanced action has the severe drawback of the deleterious effect of the steroid on skin collagen. Thus, simply increasing the strength of the steroid to make it therapeutically more effective in psoriasis would probably also produce more rapid collagen atrophy.

Future research in drugs for psoriasis must be directed at producing a steroid-like substance which affects keratinization without an adverse effect on collagen. Alternatively, basic research must try to establish more precisely the biological defect in psoriasis with a view to producing a drug to reverse or block this defect.

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Production of epidermal damage in mammalian skins by some simple aluminium compounds

A.B.G.LANSDOWN*

The British Industrial Biological Research Association,
Woodmansterne Road, Carshalton, Surrey

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SUMMARY

The responses of mouse, rabbit and pig skin to topically applied solutions of six aluminium salts have been studied in relation to their pH and the deposition of aluminium in the stratum corneum. Epidermal changes consisting of hyperplasia, microabscess formation, dermal inflammatory cell infiltration and occasionally ulceration were evident in all three species treated with aluminium chloride (10%) and nitrate (10%), but not with aluminium sulphate (10%), hydroxide (10%), acetate (10%) or chlorhydrate (10 and 25%). In skins showing pathological changes, a high degree of aluminium deposition was present in the stratum corneum and the keratin was histochemically abnormal. It contained an unusually high concentration of protein-bound sulphydryl groups and phospholipid, and fluoresced differently to normal with the congo red technique.

Although the irritant aluminium solutions were more acid than the non-irritant salts, pH was not considered to be responsible since solutions of hydrochloric acid or Universal buffer having an equivalent pH were of negligible effect.

The results of this study indicate that as a result of an interaction of the aluminium cation with epidermal keratin, the latter becomes denatured and the stratum corneum is made more permeable. The manifestation of pathological changes is attributed to the penetration of the aluminium salts through the stratum corneum and their toxic effects on the cells of the epidermis.

Aluminium compounds are extensively used in cosmetic and medicinal preparations. In these advantage is taken of their astringent, antacid, antibacterial and antiperspirant properties. The irritancy of aluminium salts to the skin varies considerably, the chloride and nitrate having greater irritant properties than the chlorhydrate, allantoinate and citrate. The latter compounds have little or no irritant effect to human skin except to particularly sensitive people (Martin *et al.*, 1966; Govette & de Navarre, 1947; Wells & Lubowe, 1964; Harry, 1963).

Two possible mechanisms have been adduced for the production of epidermal damage by solutions of aluminium compounds. They are the ability of the aluminium cation to denature epidermal keratin (Brun, Hunziker & Evdos, 1967) and the action of the free mineral acid which is formed by the hydrolysis of the compound in water (Govette & de Navarre, 1947; Heyroth, 1949; Harry, 1963).

In the present series of experiments the comparative irritancy of some aluminium salts to the skin has been studied in three species of mammals. Attention has been directed to the extent to which the pH of the solution contributes to the epidermal damage produced. In addition an attempt has been made to correlate local epidermal changes with the amount of aluminium deposited in the keratin on the assumption that such a deposition indicates an interaction with the keratin. Such an interaction could lead to protein denaturation in accordance with the well known effects of some metals on certain proteins (Joly, 1965). Previous studies have shown that as a result of denaturing epidermal keratin, an agent may elicit epidermal damage (Lansdown & Grasso, 1972).

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* Present address: Clinical Research Centre, Northwick Park Hospital, Watford Road, Harrow HA1 3UJ.

METHODS AND MATERIALS

Materials

The aluminium chloride (anhydrous), sulphate, nitrate (Analar), hydroxide and basic acetate were obtained from British Drug Houses Ltd (Poole, Dorset) and had a minimum purity of 97%. The aluminium chlorhydrate used was of the impalpable grade (Reheis Chemical Co. Inc., Chicago, Illinois).

Animals

Female mice of the TF1 strain of the original Carworth Farm Stock of albino mice (Mr A.Tuck, Rayleigh, Essex) were used in all experiments. Rabbits (New Zealand white strain, Norfolk Rabbits Ltd, Attleborough, Norfolk) and pigs (large white strain, Mr D.J.Benhill, Carshalton, Surrey) were employed in some experiments to compare the sensitivity of other species to topical applications of aluminium salts.

The mice used were randomly selected animals aged about 8 weeks. At this stage, hair growth was in the second period of telogen (resting stage). The rabbits and pigs were aged approximately 6 months at the commencement of experiments.

During the test period, all animals were maintained under a strict daily routine of 18 h light and 6 h darkness to minimize disturbances in epidermal growth due to changes in diurnal rhythm.

Determination of the local irritancy of aluminium salts

Mice, rabbits and pigs were closely shaved in the post-scapular back region with electric clippers 24 h prior to applying the test solutions. Care was taken to avoid damage to the stratum corneum but where damage was detected either the animals were discarded or fresh areas of skin were selected for test or control sites.

Test solutions (0·5 ml) were applied to 2 cm^2 areas on the backs of mice and rabbits and spread out using a clean glass rod. When pigs were used 1·0 ml of the test solutions were applied and spread out over an area of 4 cm^2. Mice and rabbits were restrained until test areas of skin had dried, and then released. Test sites remained uncovered throughout the test period.

The experiments carried out were as follows:

(1) Groups of 5 mice were treated daily for 5 consecutive days with aqueous solutions of 2·5, 5, 10 or 25% w/v aluminium chloride. Other groups of mice were treated with 10, or 25% w/v aluminium chlorhydrate in distilled water. Control animals were given equivalent applications of distilled water.

(2) Groups of 5 mice, 3 rabbits and 2 pigs were treated daily for 5 consecutive days with applications of 10·0% w/v aluminium chloride, nitrate, chlorhydrate, sulphate, hydroxide or basic acetate. Aluminium chlorhydrate was also tested at 25·0% w/v. As the hydroxide and basic acetate are not sufficiently soluble in distilled water to give 10% w/v solutions, 10% suspensions of these agents in 0·2% Tween-80 were used. Previous studies have shown that Tween-80 is not irritant to mouse skin when applied repeatedly at a concentration of 2·5% (Lansdown & Grasso, 1972). The pH of each aluminium solution was measured before use.

(3) To provide information as to whether the hydrochloric acid liberated by the hydrolysis of aluminium chloride may be responsible for the irritation seen, groups of 5 mice were treated with dilute solutions of hydrochloric acid having a pH of 2·2, 3·0 and 4·0. Other groups of mice were treated with solutions of Universal buffer (Britton & Welford, 1937) having a pH of 2·5, 3·1, 3·4 or 4·0.

24 h after the final treatment, the animals were killed by cervical dislocation (mice) or an overdose of pentabarbital sodium (Nembutal) (rabbits and pigs) and the skins examined visually with the aid of a hand lens for erythema, thickening and scaling. Representative pieces of the treated skins were taken and fixed in 70% ethyl alcohol for at least 18 h, as recommended by Jarrett & Hardy (1957). These workers preferred this fixative for skins which are to be examined using fluorescence microscopy and for ordinary light microscopy. The skins were processed and embedded in paraffin wax as usual and sectioned at 5–7 μm. Sections were stained with haematoxylin and eosin; the dye Morin, which fluoresces with a brilliant blue-green colour in the presence of aluminium and ultra-violet light (Pearse, 1960); the congo red/thioflavine T technique suggested by Jarret, Bligh & Hardy (1956) and Jarret, Spearman & Hardy (1959) for epidermal keratins; Barrnett & Seligman's (1952) technique (DDD) for protein-bound sulphydryl groups; and Baker's (1944) method for epidermal phospholipids.

RESULTS

Aluminium chloride

The changes seen macroscopically in mouse skins treated with aluminium chloride consisted of erythema, thickening and scaling. Applications of 25% solutions produced changes which were evident after only 2 days, and by 4 days the damage was severe in all animals of the group. 10% aluminium chloride caused severe erythema, thickening and scaling after 5 days treatment. In mice treated with the lower concentrations, the changes were appreciably less marked, such that the skins of animals given 2·5% did not differ appreciably from the controls treated with distilled water only.

The histological changes seen in mouse skins treated with aluminium chloride are given in Table 1.

TABLE 1. Irritancy of aluminium chloride and chlorhydrate to mouse skin

	Hyperkeratosis	Acanthosis	Microabscess in epidermal keratin	Aluminium deposits in keratin
Aluminium chloride				
25%	++++*	+++	++++	++++
10%	+++	++++	+++	+++
5%	++	+++	–	+
2·5%	±	–	–	±
Aluminium chlorhydrate				
25%	–	–	–	–
10%	–	–	–	–

++++, severe hyperplasia with focal ulceration; +++, marked hyperplasia with epidermis 3–5 times thicker than usual; ++, moderate hyperplasia; +, slight hyperplasia; ±, epidermis normal for the most part but contains few small foci of hyperplasia; *, the pathological changes did not vary appreciably in severity in animals of any group; –, normal in appearance.

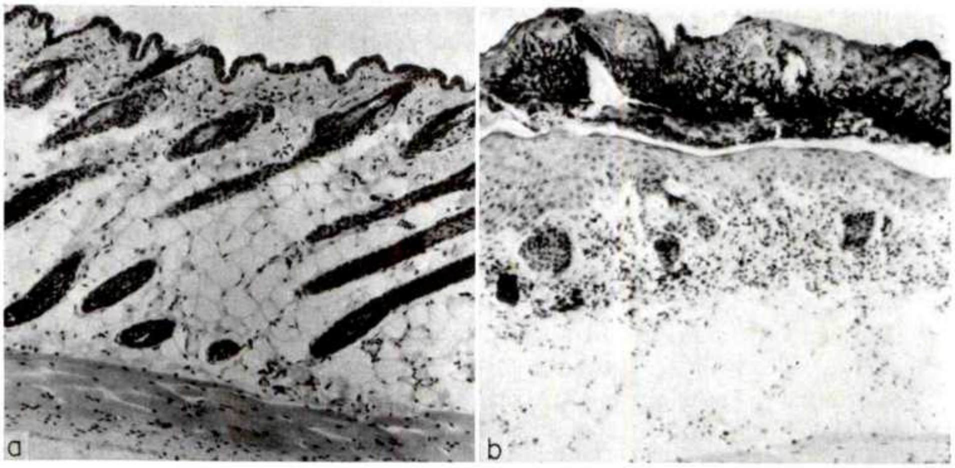

FIGURE 1. (a) Transverse section of normal mouse skin (H & E, × 6·3 objective). (b) Section of mouse skin treated with 10% aluminium chloride to show hyperkeratosis, epidermal microabscesses and acanthosis with dermal inflammatory cell infiltration (H & E, × 6·3 objective).

Skins treated with 25% solutions exhibited pronounced epidermal hyperkeratosis, acanthosis with marked inter- and intra-cellular oedema in the epidermis. Many foci of leukocytic infiltration and microabscess formation were evident in the keratin layer (Fig. 1) and in a number of skins ulceration was present. Dermal changes consisted of oedema and increased leukocyte infiltration which were particularly pronounced in the dermo-epidermal regions near to areas of severe acanthosis and foci of ulceration. In most skins examined vascular engorgement was present in the superficial blood vessels.

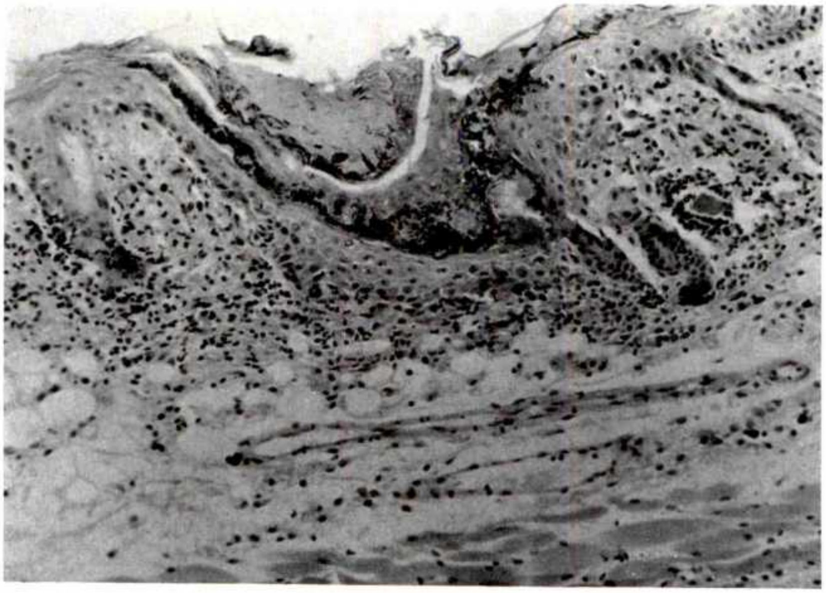

FIGURE 2. Section of mouse skin treated with 10% aluminium chloride to show microabscesses, keratin, cell debris and exudate in the dilated ostium of a hair follicle (H & E, × 6·3 objective).

A further lesion identified consisted of a mass of keratin, cell debris and tissue exudate in the ostia of hair follicles (Fig. 2). In several instances these ostia were dilated and the distal epithelium was frequently acanthotic. Associated with the marked acanthosis seen in most skins given 25% aluminium chloride was greatly thickened stratum granulosum.

The pathological changes in the skins of mice treated with 10% aluminium chloride were less severe than those seen with the 25% solution and fewer foci of ulceration were apparent. Micro-abscesses were present in the skins of all animals of the group and dermal oedema, inflammatory cell infiltration and vascular engorgement were prominent. 5% solutions produced slight to moderate hyperplasia in most skins examined but at this concentration foci of inflammatory cell infiltration in the keratin were rarely seen and dermal changes were minimal. Mouse skins treated topically with 2·5% solutions did not differ from control skins treated with distilled water only.

In rabbits and pigs treated topically with applications of 10% aluminium chloride, the visual and histological changes (Table 2) were of a similar type to those observed in mice. However, the degree of hyperkeratosis was less in pig skins than mouse or rabbit skins.

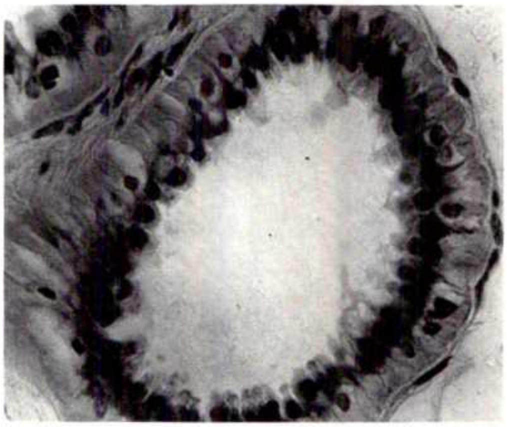

FIGURE 3. Transverse section of a secretory tubule of an apocrine sweat gland from pig skin treated with 10% aluminium chloride. The cytoplasm in many of the cells is in the process of being pinched off into the lumen (H & E, × 40 objective).

In the pig skins examined after exposure to 10% aluminium chloride the apocrine sweat glands were active in many areas and histologically were similar to those seen in control skins. Small droplets of cytoplasm were seen in the process of being extruded into the duct lumens (Fig. 3). A similar condition has been reported previously in anatomical investigations of pig skin (Winter, 1966). Thus it would seem that treatment with 10% aluminium chloride does not adversely affect the functional capacity of the sweat glands.

Aluminium chlorhydrate

No visible or microscopic changes were evident in the skins of mice rabbits or pigs treated daily with solutions of 10 or 25% aluminium chlorhydrate in water. As with aluminium chloride treatment, the sweat glands in pig skin were functional after treatment with aluminium chlorhydrate.

Comparative irritancy of other aluminium salts with the chloride

The response of the three species to topical applications of the aluminium salts is given in Table 2.

TABLE 2. Epidermal changes in mice, rabbits and pigs treated with solutions of aluminium salts

	Hyperkeratosis	Acanthosis	Microabscesses	Aluminium in keratin
10% aluminium chloride				
mouse	+	+	+	+
rabbit	+	+	+	+
pig	+	+	+	+
10% aluminium chlorhydrate				
mouse	—	—	—	—
rabbit	—	—	—	—
pig	—	—	—	—
10% aluminium nitrate				
mouse	+	+	+	+
rabbit	+	+	+	+
pig	+	+	±	+
10% aluminium sulphate				
mouse	—	—	—	—
rabbit	—	—	—	—
pig	—	—	—	—
10% aluminium hydroxide				
mouse	—	—	—	—
rabbit	—	—	—	—
pig	—	—	—	—
10% basic aluminium acetate				
mouse	—	—	—	—
rabbit	—	—	—	—
pig	—	—	—	—

+, changes clearly visible histologically and visually; ±, changes slight or of questionable validity to treatment given; —, normal in appearance.

10% aqueous solutions of aluminium sulphate, hydroxide and acetate did not produce visual or microscopic changes after 5 days' treatment, neither did they alter the activity of the porcine sweat glands.

In contrast, aluminium nitrate when applied at 10% in water produced pathological changes in mouse and rabbit skins which were similar to those evoked by 10% aluminium chloride. Pig skins treated with aluminium nitrate tended to be less severely affected than either mouse or rabbit skins. The hyperplasia was less and dermal changes minimal but microabscesses were occasionally present in the keratin layer. Although some areas of dermal inflammatory cell infiltration were present this effect was not marked. The responses of the three species studied to aluminium nitrate were less marked than those seen with aluminium chloride treatment.

pH *as a factor in the production of epidermal damage by aluminium salts*

The relationship between the pH of the aluminium solutions and the irritancy produced is given in

TABLE 3. pH of solutions of aluminium salts and its relationship to irritation and aluminium deposition in epidermal keratin

	pH	Irritancy	Aluminium deposition in epidermal keratin
Aluminium chloride (10% aq.)	2·3	+ + +	+ + +
Aluminium chlorhydrate (25% aq.)	4·2	—	—
Aluminium nitrate (10% aq.)	2·4	+ + +	+ + +
Aluminium sulphate (10% aq.)	3·0	—	—
Aluminium hydroxide (10% suspension in Tween-80)	7.2	—	—
Basic aluminium acetate (10% suspension in Tween-80)	4·8	—	—

+ + +, severe; + +, moderate; +, slight; —, normal.

Table 3. The salts with the most irritant effects, the chloride and nitrate, exhibited a pH of less than 2·5. The less acid solutions of aluminium sulphate, hydroxide, chlorhydrate and basic acetate having pH levels of greater than 3·0 were all relatively non-irritant in the three species, under the conditions used.

When dilute solutions of hydrochloric acid were applied daily to mouse skins for 5 days, cutaneous reactions were present only in those skins receiving the solution having a pH of 2·2. One animal showed slight erythema on the fifth day of treatment. Histologically, slight hyperplasia was present in all animals of the group but in no instance was the condition pronounced. The stratum spinosum was seldom more than twice its normal thickness and the dermal inflammatory cell infiltration was not appreciably different from control skins. No changes were evident in the skins of mice given universal buffer at pH 2·5–4·0.

Aluminium deposition in the epidermis

Deposits of aluminium in the epidermis as indicated using the Morin fluorescence technique were seen in the surface layers of the stratum corneum of those animals treated with aluminium chloride or nitrate (Tables 1–3). In general the greatest deposition of aluminium was present in those skins exhibiting pronounced hyperkeratosis and acanthosis (Fig. 4). In these skins foci of aluminium fluorescence were occasionally present in the pilosebaceous follicles and in the dermo-epidermal region.

In the stratum corneum, the deposits of aluminium were frequently seen in the vicinity of the pilosebaceous follicles and on occasions appeared to form 'plugs' of the type described by Brun *et al.* (1967) (Fig. 5). In the present work, plugs were only present in those skins treated with aluminium chloride or nitrate.

Histochemical observations on the nature of the keratin in mouse skins treated with aluminium chloride

In normal mouse skin, the epidermal keratin fluoresces with a brilliant red coloration when stained with congo red and irradiated with ultra-violet light. Hair keratin fluoresces blue. In the hyperkeratotic regions of skins treated with aluminium chloride only a narrow region on the surface of the keratin

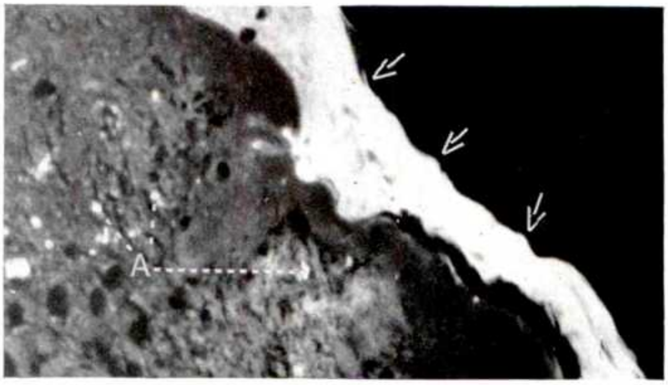

FIGURE 4. Mouse skin treated with 10% aluminium chloride showing aluminium-fluorescence (arrowed) in stratum corneum and in the dermis (A) (Morin, × 6·3 objective).

fluoresced red, the remainder of the epidermal keratin showing a dull blue-grey colour. According to Jarrett *et al.* (1959) this change in fluorescence is indicative of an alteration in the constitution of the keratin.

In skins stained for free sulphydryl groups by Barrnett & Seligman's DDD method, normal keratin stained pink with hair keratin a deep pink colour, indicating a high concentration of sulphydryl at this site. The hyperkeratotic and parakeratotic regions of mouse skin exhibited deep pink or occasionally blue staining in the stratum corneum, indicating a considerable increase in sulphydryl groups.

The abnormal nature of the keratin in the hyperkeratotic regions of aluminium treated skins was further demonstrated using Baker's technique for phospholipids. Whereas in normal skins blue stain-

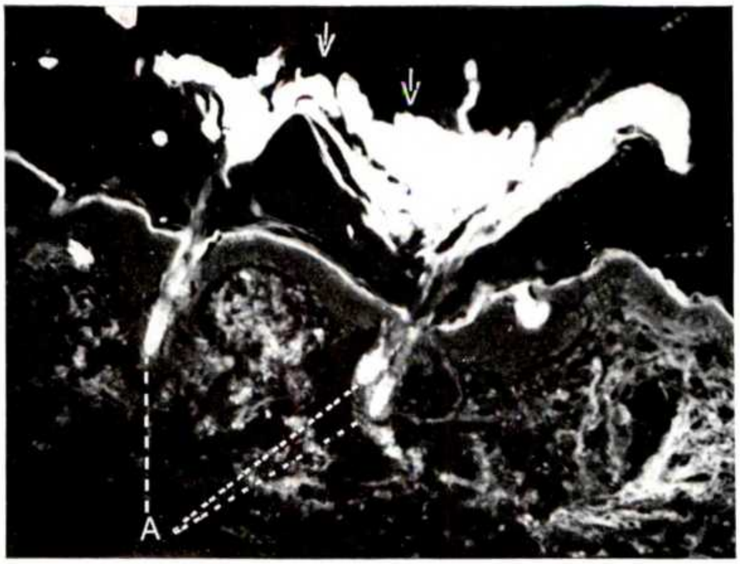

FIGURE 5. Mouse skin treated with 10% aluminium chloride showing aluminium-fluorescence concentrated in the keratin in the vicinity of the pilosebaceous follicle (arrowed) and in the follicles (A) (Morin, × 6·3 objective).

ing for phospholipids was restricted to a small zone surrounding the hair roots, with no staining evident in the epidermal keratin, in hyperkeratotic skins most of the stratum corneum was stained intensely blue and only a narrow region on the surface remained colourless.

In the majority of skins examined the zones where phospholipid and sulphydryl group concentrations were high and where the keratin fluoresced blue with congo red, hyperkeratosis was severe. Also when these skins were stained with Morin, it became evident that these areas contained high deposits of aluminium.

DISCUSSION

The epidermal damage caused by topical applications of aluminium salts appears to be related directly to the deposition of aluminium in the keratin. Although the two salts which proved to be irritant gave more acidic solutions in water than the non-irritant compounds, the pH of the test solution does not appear to contribute to the pathological lesions observed. It would seem that the pH is important only in so far as it creates an environment which favours the deposition of the aluminium in the epidermal keratin.

The results of the present study carried out under *in vivo* conditions contrast with those obtained by Lyon & Klotz (1958) who investigated the uptake of aluminium *in vitro* from solutions of aluminium chloride and sulphate by keratins extracted from human and animal skins. These authors showed that aluminium is taken up well irrespective of whether chloride or sulphate is used, if the keratins were extracted with lipophilic solvents. No aluminium uptake was observed with either salt if lipophilic solvents were not employed. Lyon & Klotz (1958) believed that skin lipids interfered with the uptake of aluminium. This explanation does not seem to be consistent with our *in vivo* work, since no procedure aimed at removing the skin lipids was applied prior to the application of aluminium chloride or nitrate to the skin where aluminium uptake was pronounced.

The interaction between metal salts and proteins was discussed by Joly (1965) who referred to the aggregation of serum albumin in solutions of aluminium chloride. He noted that such an interaction may be accompanied by a denaturation of the protein. It is possible that the uptake of aluminium by the keratin may reflect an interaction between the metal and the keratin leading to the denaturation of the latter.

The aluminium–keratin interaction with the probability of keratin denaturation has possible implications in the manifestation of epidermal damage. Wood & Bettley (1971) recently reported that in skins treated with anionic surfactants which denatured the epidermal keratin, the stratum corneum is made more permeable to topically applied substances. This implies that the underlying epidermal cells are exposed to injury by the agent which is responsible for denaturing the keratin. Cellular injury brought about by this route may in turn give rise to the pathological changes of hyperkeratosis, acanthosis, parakeratosis and ultimately ulceration. Although existing evidence points to the fact that little aluminium from topically applied solutions of its salts penetrates deeper than the most superficial layers of the stratum corneum, some does reach the dermis (Blank, Jones & Gould, 1958). It is conceivable that this low concentration is sufficiently toxic to bring about the changes seen histologically.

The histochemical techniques employed demonstrated a marked difference in the phospholipid content and available sulphydryl groups in the epidermis showing histological changes consistent with hyperkeratosis and parakeratosis. These changes have been described earlier by Jarrett *et al.* (1959) in psoriasis, warts and pressure keratinization and have been interpreted as abnormal keratinization. It would appear that some similar abnormal keratinization is occurring in the skin of mice treated with aluminium chloride and aluminium nitrate. It is perhaps of some significance that although these changes were quite marked in areas of pronounced hyperkeratosis they were also seen in areas of skin which showed doubtful histological evidence of hyperkeratosis.

In this series of experiments, aluminium chlorhydrate did not produce epidermal damage, presumably because the aluminium was not available in a form which could interact with the keratin. However, since this compound has been reported to cause adverse reactions in 'particularly sensitive people' one may speculate that some aluminium–protein interaction is possible if the physiological state of the skin differs from normal. Changes in the acidity of the skin, its state of hydration or the level of sebaceous lipids present on the surface may be contributory to the production of the rare instances of irritancy seen.

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Pemphigus controlled by sulphapyridine

P.P.SEAH, LIONEL FRY, R.J.CAIRNS* AND M.FEIWEL

St Mary's Hospital, London, W.2, and *St Bartholomew's Hospital, Rochester, Kent

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SUMMARY

A patient with an 11-year history of a generalized blistering eruption is described. The eruption was completely controlled within 1 week of taking sulphapyridine and recurred within 4 days of withdrawal of this drug. On account of these observations a diagnosis of dermatitis herpetiformis was made. Histological, immunological (and small intestinal) studies now show that this patient had pemphigus.

Dapsone and sulphapyridine both control the eruption of dermatitis herpetiformis. The response is so dramatic that a therapeutic trial of either drug is often used in the diagnosis of this condition. There have been reports of four patients (Floden & Gentele, 1955; Doepfner, 1960; Winklemann & Roth, 1960) in whom the diagnosis of dermatitis herpetiformis had been queried, as these patients had a blistering eruption which was controlled by sulphonamides though the histology of the lesions showed the blisters to be intraepidermal. We report a patient whose blistering eruption was controlled by sulphapyridine and, as a result, was diagnosed as having dermatitis herpetiformis (even though histologically the blister was intraepidermal), but now immunological studies have shown the patient to have pemphigus and no evidence of dermatitis herpetiformis.

CASE REPORT

History

The patient was a female Caucasoid aged 43. 11 years ago, at the age of 32, she developed an irritating eruption, which she described as 'spots', on her shoulders and abdomen. There were no blisters at this time. Her general practitioner initially diagnosed ringworm infection and, subsequently, pityriasis versicolor. Her eruption failed to respond to a number of ointments and she developed blisters a few weeks later on the trunk and shoulders; she was then referred to St Bartholomew's Hospital, Rochester. A clinical diagnosis of dermatitis herpetiformis was made and a biopsy carried out. The biopsy was reported by the Histopathology Department at St John's Hospital for Diseases of the Skin as follows: 'There are two intra-epidermal bullae filled with eosinophils and one bulla shows acantholysis, which does not imply that the case is pemphigus vulgaris'. The patient was initially treated as for dermatitis herpetiformis with dapsone, 200 mg/day, and the eruption cleared within 2 weeks. However, 3 months later, whilst she was still taking this dose of dapsone, the rash returned. When the dose of dapsone was increased to 300 mg/day she felt unwell and became 'blue'. This was shown to be due to methaemoglobinaemia. The dapsone was stopped and she was given a 6 week course of systemic steroids with little improvement. Following this she was given sulphapyridine 1·5 g/day and this controlled the eruption. She has now been taking this drug for 10 years, although the dose needed for control of the eruption is now 3·0 g/day.

In 1972 she was referred to the dermatitis herpetiformis clinic at St Mary's Hospital for reassessment and possible treatment by a gluten-free diet. Physical examination showed no abnormality apart from numerous small scars. Sulphapyridine therapy was stopped, and 3 days later the patient developed a vesicular eruption on the limbs and trunk. The rash subsided within a week of recommencing the sulphapyridine, and on stopping this drug a second time she again developed blisters within 4 days.

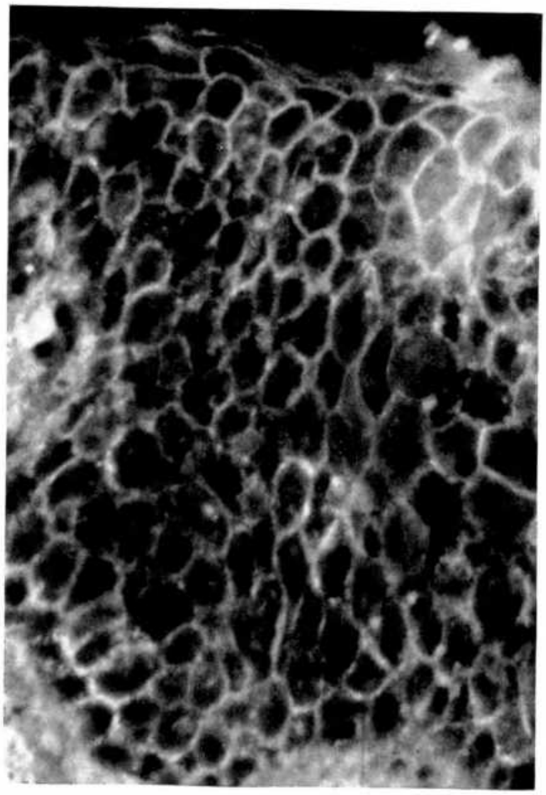

FIGURE 1. High power view of positive epidermal intercellular fluorescence. Section of skin taken adjacent to bullous lesion stained with FITC–sheep–anti–human IgG for direct immunofluorescence (× 800).

Investigations

Haematological. Hb 13·1 g/100 ml, WBC 7100/mm^3, serum folate 8 ng/ml, red cell folate 506 ng/ml, serum vitamin B_{12} 564 pg/ml, serum iron 195 μg/100 ml, bone marrow biopsy normal apart from no stainable iron.

Gastro-intestinal. Faecal fat excretion 0·7 g/day, small intestinal biopsy: macroscopic, fingers and leaves; microscopic, normal villi with columnar epithelium; epithelial lymphocyte count, 253/thousand epithelial cells.

Skin biopsy. This showed an intradermal blister containing acantholytic cells and eosinophils. The level of the blister is predominantly subcorneal.

Immunological (direct immunofluorescent studies). These studies were carried out as described by Seah *et al.* (1972).

Class specific fluorescein isothiocyanate (FITC) conjugated sheep-anti-human IgG, IgM and IgA (Wellcome Reagents) and FITC-goat-anti-human $\beta 1_c/\beta 1_a$ (complement C_3) (Hylands) were used. Controls were provided by incubating the patient's skin sections with FITC conjugated sheep-anti-rabbit anti-serum (Wellcome Reagents) and by incubating sections of skin from a normal person with the same conjugates. Specimens were examined on a Reichert 'Diapan' microscope with a quartz iodine light source, a Balzer FITC 3 primary interference filter and a Wratten 12 secondary filter.

Strong staining of the intercellular spaces in the epidermis was seen with anti-IgG (Fig. 1). No IgM, IgA or $\beta 1_c/\beta 1_a$ deposits were detected in the dermis or epidermis. There was no evidence of basement membrane staining. No immunofluorescent staining was seen with the controls.

Indirect immunofluorescent studies. A polyvalent FITC sheep-anti-human immunoglobulin conjugate (Wellcome Reagents) was used for these tests.

Intercellular antibody (Beutner & Jordon, 1964) was detected at a titre of 1 : 10. There was a positive anti-nuclear factor at a titre of 1 : 80. No basement membrane (Beutner *et al.*, 1965), reticulin, smooth muscle, mitochondrial or gastric parietal cell antibodies (Seah *et al.*, 1971) were detected.

DISCUSSION

This report shows that the patient has pemphigus and not dermatitis herpetiformis, and the disorder is controlled by sulphonamides. The report made 10 years ago by the histopathologist, that the findings do not imply pemphigus, cannot be accepted. The presence of eosinophils in the involved epidermis in patients with pemphigus has been stressed by Emmerson & Wilson-Jones (1968) and cannot, as an isolated finding, be considered to favour a diagnosis of dermatitis herpetiformis.

Certainly in the past, and in the majority of dermatology departments even now, the response of a blistering eruption to sulphonamides and/or dapsone has been taken to imply a diagnosis of dermatitis herpetiformis. However, this assumption must now be seriously questioned, as although the rash of dermatitis herpetiformis does respond to dapsone and sulphonamides, there are reports of other dermatoses, particularly vasculitis and erythema elevatum diutinum (Johnston, 1964; Kalkoff, 1968; Vollum, 1968) responding to these drugs. There have been reports of four patients similar to ours with an eruption of histologically intra-epidermal blisters, controlled by sulphonamides (Floden & Gentele, 1955; Doepfner, 1960; Winklemann & Roth, 1960). All these three reports queried whether these four patients had dermatitis herpetiformis or pemphigus.

Over the last few years definite immunological criteria for diagnosing pemphigus and dermatitis herpetiformis have evolved. The previous reports of this rare group of patients with intra-epidermal blisters showing acantholysis on microscopy, yet responding to sulphonamides have not included immunological findings (although Dobmeier, Mitchell Sams & Beutner (1971) have reported a patient with circulating antibodies characteristic of pemphigus whose eruption responded to sulphonamides, but no intra-epidermal blisters were ever found on microscopy). Our patient showed both *in vivo* intercellular antibodies in the epidermis (as described by Beutner *et al.*, 1965) and circulating antibodies to intercellular cement substance of epidermal cells (as described by Beutner & Jordon, 1964), which may be considered necessary criteria for the diagnosis of pemphigus. Moreover, there were no IgA deposits in the dermal papillae of the uninvolved skin, such as are present in patients with dermatitis herpetiformis (Van der Meer, 1969; Chorzelski *et al.*, 1971; Seah *et al.*, 1972). Further evidence that this patient does not have dermatitis herpetiformis comes from the investigation of her gastrointestinal tract and folate status. 95% of patients with dermatitis herpetiformis show abnormality of the small intestinal epithelium, which is due to gluten sensitivity (Fry *et al.*, 1972). The most sensitive test for gluten sensitivity is the degree of lymphocytic infiltration of the small intestinal epithelium (Fry *et al.*, 1972). In our patient the macroscopic appearance of her small intestine was normal and her lymphocyte count was 253. This is a high normal figure well below the range of 451 ± 70 found in patients with dermatitis herpetiformis. In addition, 90% of patients with dermatitis herpetiformis have a low serum folate (Fry *et al.*, 1972), and our patient had a normal serum level.

Thus, this is a further report of the control by sulphonamides of the skin eruption of a patient with pemphigus, and the first report of such a patient in whom the diagnosis of pemphigus has been established by immunological studies. The mechanism of action of the sulphonamides in this disorder remains unknown, but we presume it is a block of an immunological reaction. Unfortunately, at the present time, there are no further investigations which can distinguish this rare group of patients with pemphigus, whose rash is controlled by sulphonamides, from the majority who require other more potent and dangerous drugs.

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Allopurinol in psoriasis—a double-blind study

ELEASAR J.FEUERMAN AND MARK A.NIR

Department of Dermatology, Beilinson Medical Center,
Tel Aviv University Medical School, Petah Tiqva, Israel

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SUMMARY

In this double-blind cross-over study allopurinol was found to be no more effective than a placebo in the treatment of psoriasis.

The report of Viglioglia *et al.* (1970) on the effectiveness of allopurinol in the treatment of psoriasis prompted us to undertake this double-blind cross-over study on the effect of allopurinol and a placebo in psoriatic patients.

MATERIAL AND METHODS

Sixteen patients aged 32–63 years were initially included in the study. Thirteen of them (eight males and five females) completed the study. The duration and severity of the disease, nail involvement, initial uric acid values and concurrent disease and its treatment are summarized in Table 1. None of the patients suffered from psoriatic arthritis.

Patients with a wide spectrum of involvement were included in the study. Some were chosen because of severity and prolonged duration of the disease and lack of response to topical therapy. The nature of the study was explained to the patients and their consent was obtained.

100 mg tablets of the 'Urical' brand of 4-hydroxypyrazole (3, 4-D) pyrimidine (allopurinol)* were randomized with tablets of placebo of identical appearance. There were two treatment periods, each lasting 8 weeks. At the beginning of each period the patients were hospitalized for 2 weeks. They were given 2 tablets three times daily; i.e. during the actual treatment period they received 600 mg of allopurinol daily. The investigators had no knowledge of whether allopurinol or the placebo was being administered at any given period.

On day 57 of the trial, when patients were admitted for the second 2-week period of hospitalization, they were switched to the alternate batch without their knowledge. During the hospitalization periods physical examination and routine laboratory tests were performed, including blood counts and liver and kidney function studies. Uric acid determinations were performed only at the beginning of the first period, to prevent the code from being broken. The extent and severity of the lesions were assessed and recorded at weekly intervals throughout the study, and were also documented photographically at the beginning and end of each period.

* Thanks are due to Mr Tomer, Plantex Pharmaceuticals, for kindly supplying the identically appearing tablets of Urical^R and placebo.

TABLE 1. Clinical details

No.	Sex	Age	Duration (years)	Clinical picture	Nails	Initial uric acid	Other diseases and treatment
1	F	46	15	Generalized	Pits	4·5	
2	M	40	23	Stable, extensors, trunk	Pits	4·9	
3	F	48	6	Active, extensors, trunk	No	7·0	Diabetes mellitus (diet) hyperlipaemia
4	F	63	22	Active, generalized	No	5·2	Mild diabetes mellitus
5	M	43	15	Very extensive, confluent	No	4·9	
6	M	61	10	Generalized	No	7·2	Bronchial asthma, hepatomegaly
7	M	57	10	Generalized	Severe	6·0	
8	M	43	11	Stable, extensors, trunk	Some	5·4	
9	M	41	15	Quite extensive	Pits	5·7	Lues, seropositive (penicillin 1,200,000 × 10)
10	M	32	13	Quite extensive	No	5·7	Bronchial asthma (Aminophyllin)
11	M	56	20	Quite extensive	Pits	6·1	
12	F	45	21	Quite extensive	No	3·0	Epilepsy (hydantoin)
13	F	54	12	Extensors only	Pits	4·2	

On discharge at the end of the 2-week period of hospitalization the patients were given a quantity of tablets sufficient for one week. This supply was then replenished at weekly intervals. On days 21 and 28 of both periods, nail measurements were performed according to the method of Dawber (1970). The third finger of the left hand was scored and measured using a Vernier caliper accurate to 0·1 mm. The study periods of individual patients were distributed between April 1971 and April 1972, so as to minimize the possible effect of climatic changes. No topical treatment was given during the whole study period.

RESULTS

When the code was broken it was found that six patients received allopurinol during the first period and seven during the second period. The results are given in Table 2.

In two patients, both placebo and allopurinol were ineffective. Four patients showed considerable improvement while on allopurinol, and three patients showed an improvement while on placebo. Comparing their state with that in the preceding period, two patients were found to have become worse on the placebo and three patients to have become worse on allopurinol.

Of the four patients who improved on allopurinol, two had received the drug during the first period, of which one started to clear after 2 weeks of therapy and the other after 4 weeks. In both these cases there was progressive improvement, the first becoming lesion-free at the end of the eleventh week and the second at the end of the thirteenth week, despite the change-over to placebo after the eighth week. Of the three patients who improved on the placebo, two had received it in the first period. In both, the lesions started to regress by the end of the third week. One was completely free of lesions by the

TABLE 2. Results of double-blind study

No.	Allopurinol	Placebo
1	No change	No change
6	No change	Moderate
7	Excellent	Continued remission
8	No change	Worse
11	Excellent	Continued remission
13	No change	Worse
	Placebo	**Allopurinol**
2	No change	Slightly worse
3	No change	No change
4	No change	Worse
5	Excellent	New lesions at 6 weeks
9	Good	No change
10	Worse	Good
12	No change	Good

end of the seventh week, and continued in remission during the allopurinol period, at the end of which several new lesions appeared. The other patient was almost clear of lesions by the seventh week, at which time a few new lesions appeared; following the change to allopurinol this patient's condition remained static.

Nail growth

Since there were no significant differences in the average daily nail growth rates as measured on day 21 and day 28, data concerning the latter only are given. Nail growth during the allopurinol periods averaged 0·11 mm/day (range 0·06–0·16) as compared with an average of 0·12 mm/day (range 0·07–0·17) on placebo. This difference is not statistically significant.

Side effects

None of the known adverse reactions to allopurinol was observed among the thirteen patients who completed the course of treatment. Of the three patients who did not complete the study, one developed erythroderma while on the placebo. The second developed a drug eruption while on allopurinol, and the third patient was lost to follow-up.

DISCUSSION

The results of this study demonstrate that allopurinol was no more effective than a placebo in the treatment of psoriasis.

Although the sample was small, we feel that valid conclusions can be reached. Had there been more 'placebo reactors' (Lasagna *et al.*, 1954) in the control group than in the group receiving allopurinol, we would not have expected a complete lack of response in four of the seven patients initially on the placebo. Moreover, even if there were no distinct rebound phenomena, some worsening of the condition might have been expected in the two patients who had received allopurinol first and improved, barring a very protracted 'carry-over' effect (Rinzler *et al.*, 1953). We are thus inclined to believe that the absence of a rebound phenomenon, as stressed by Viglioglia *et al.* (1970), is a feature of the natural course of psoriasis, as in our case 6, who commenced to clear rapidly on the placebo, and in whom new lesions appeared at the end of the allopurinol period.

Emotional factors are implicated in the natural history of psoriasis (Obermayer & Borelli, 1970; Baughman & Sobel, 1971) and are of major importance in the mechanism of placebo action (Lowenthal, 1970). The high rate of improvement in our placebo group is therefore not surprising. By the same token, we would expect an even higher improvement rate in our allopurinol group, had the drug possessed any therapeutic activity beyond the placebo effect.

This study serves well to stress the need for carefully designed experiments for the evaluation of anti-psoriatic drugs, and explains to some extent the alleged successful results of various therapeutic and dietetic regimens. On the other hand, it also points towards the possibility of intelligent exploitation of the placebo effect as an adjunct to the management of psoriasis.

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Comment

THE MOLECULAR BASIS OF CONNECTIVE TISSUE PATHOLOGY

Connective tissue is widely distributed in all multicellular organisms. It ensures the support of the various parenchymal cells and allows exchange between them. It is made of several types of fibrils resistant to traction dispersed within an interfibrillar permeable ground substance. The three fibrous proteins which polymerize to form the fibrils represent a unique class of biological compounds. Alterations in their chemical structure result in a pathology mostly related to abnormal mechanical properties as illustrated in man and animals. Some of these diseases can now be defined in terms of molecular pathology.

The respective physical properties of the different connective tissues in mammals depend on variations around a basic architectural organization. The synthetic activity of fibroblasts or cells of a similar line, and perhaps some parenchymal cells, produces protein monomers whose polypeptides share common chemical and biological properties: collagen, elastin and some structural glycopolypeptides of the basement membranes. All these proteins associate into polymers outside the cells, in the extracellular fluid. All three depend for their structure and function on similar biochemical processes.*

Between polypeptide synthesis, using the classical machinery of protein synthesis, and the extrusion of the molecules from the cells, the side chain of some amino acids undergoes chemical modifications through the activity of specific enzymes. In precisely determined sequences, prolyl residues are hydroxylated to hydroxyproline by prolyl-protocollagen hydroxylase, and lysyl residues transformed to hydroxylysine by lysyl-protocollagen hydroxylase. Both hydroxylases use molecular oxygen and need cofactors to express their full activity, α-ketoglutarate, Fe^{++}, and ascorbate. These reactions occur in collagen and basement membrane collagen-like glycoproteins. Prolyl hydroxylation alone occurs also in elastin. Subsequently, two sugar transferases (galactosyl- and glucosyl-) bind to specifically located hydroxylysyl residues galactose alone or a galactosyl-glucose disaccharide. In collagen and elastin, and perhaps also in basement membrane glycoproteins, specific lysyl residues are oxydized to their aldehyde (allysine) by lysyl oxydase, using Cu^{++} as a cofactor. These aldehydes are precursors of interpolypeptides, covalent bonds forming progressively in the extracellular fluid, and are required for the mechanical properties of the fibres. In the extracellular space, collagen is organized into polymers, assembled in fibrils, and joined together into bundles. The collagen monomer forming the fibrils is made of three coiled polypeptides (α-chains) assembled into a rigid rod. At the time of its secretion from the cells in the form of a precursor, it is longer than the monomer in the fibrils by the presence of a polypeptide extension at the N-terminal end of its constitutive α-chains. These extensions, involved in polymerization, are split in the extracellular space by a specific enzyme, procollagen peptidase, requiring Ca^{++} as a cofactor. Degradation of the collagen fibres is initiated by collagenase, a specific enzyme also requiring Ca^{++} as a cofactor and resulting from the activation of procollagenase.

Other compounds, such as proteoglycans and structural glycoproteins, also secreted by the fibroblast, most probably interact with the fibrous proteins to condition their structural organization and function. As an example, the elastic fibres are made of filaments of acidic polypeptides in which elastin is later polymerized.

During growth, development, and healing, normal or pathological, the changes in architecture of the collagen framework result from the intricate process of remodelling. Precisely timed and located degradation and synthesis of the collagen fibres allow a progressive modification of the shape of the tissue without impairing its mechanical properties. This remodelling, most obvious during growth, is expressed in man and animals by the elevated urinary excretion of peptide-bound hydroxyproline during infancy and at puberty. The metabolism of the elastic fibres is more reduced, if present, in the adult. Little is known about metabolism in basement membranes.

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* Only a few specific references are provided in the text. Most of the pertinent bibliography not included can be found in a comprehensive recent review by Gallop, Blumenfield & Seifter, 1972.

Correct mechanical properties of the various connective tissues result from the proper assembly of the required amount of all components secreted in the desired environment. On such an operational basis, pathology can be defined in terms of molecular defects. Alterations occurring in the proteins of the connective tissues can be divided into two major categories: structural and control defects. The first would result from an alteration in the structure of the molecules. The second would occur through an abnormal balance between synthesis and degradation. Both could be produced by two different mechanisms: alteration in the structure of the genetic material (i.e., inheritable diseases), or alteration imposed on otherwise normal gene products by an event leading to abnormal chemical reaction(s).

Among the structural defects of genetic origin, two diseases, the first in the calf, dermatosparaxis, and the second in man, lysyl-protocollagen hydroxylase deficiency, are finding satisfactory explanations at the molecular level.

Dermatosparaxis of the calf is characterized by an extreme fragility of the skin, easy wounding, and oedema occurring immediately after birth. This condition is transmitted in a pattern consistent with autosomal recessive inheritance. The skin of the dermatosparactic calves is composed of abnormal collagen fibres polymerized into ribbons instead of cylindrical fibrils, and lacking their parallel packing. The collagen molecules forming such defective polymers are larger than normal and similar to procollagen, the precursor of collagen from which the N-terminal polypeptide extensions have not been removed (Lenaers *et al.*, 1971). The defect is due to the inactivity of procollagen peptidase, the excising enzyme (Lapière, Lenaers & Kohn, 1971). The impaired mechanical properties are probably related to the steric hindrance introduced by the uncleaved polypeptide preventing the cross-linking reaction through the lysyl aldehyde near the amino-end of the α-chains (Bailey & Lapière, 1973).

Lysyl-protocollagen hydroxylase deficiency expresses itself as abnormal mechanical properties of dermis, bone, and tendon, as in the Ehlers–Danlos syndrome. The condition has been detected in two sisters, and the abnormal mechanical properties of their connective tissue related to a striking reduction of hydroxylysine in their collagen (Pinnel *et al.*, 1972). This results from a reduced amount of the specific enzyme, lysyl-protocollagen hydroxylase. The presence of the defect in two sisters, and the reduction of the enzyme in one parent, strongly suggest an autosomal recessive inheritance (Krane, Pinnell & Erbe, 1972). The defect is directly related to the formation of the crosslinks, since hydroxylysine (or its aldehyde) is involved in the chemical reaction leading to the formation of covalent bonds between collagen molecules.

In view of the preceding observations, it is quite obvious that the Ehlers–Danlos syndrome represents a heretogeneous population of patients in terms of molecular defects in collagen. In this syndrome, the collagen fibres seem normal in the electron microscope, but the fibres associate in altered bundles, as seen in the scanning electron microscope. Not only could defective enzyme produce such an alteration but also limited, and still hypothetical, modification of the primary structure of the molecules. For example, a mutation introducing a different amino acid at the cross-linking site, or a bulky or charged amino acid in a neighbouring position, might interfere with the chemical reaction involved in crosslinking, and produce a collagen molecule able to polymerize but unable to crosslink correctly. This hypothesis is supported by the recent observation that an abnormal pattern of crosslinking precursors and products exist in the skin of an Ehlers–Danlos patient (Mechanic, 1972).

A structural defect of genetic origin in the proteins forming the elastic fibres might be responsible for pseudoxanthoma elasticum. Elastin seems normal, but the microfilaments of acidic proteins directing the polymerization of elastin are altered (Ross, Fialkow & Altman, 1972).

Other diseases might exist which are associated with a structural defect of genetic origin. We have observed recurrent extensive spontaneous ulceration and slow healing in a young Italian girl excreting tremendous amounts of collagen metabolites in the urine. The largest proportion of the excreted peptides were of an amino acid sequence compatible with collagen, except for reduction in the hydroxylation of proline. Administration of large amounts of the cofactors of prolyl-protocollagen hydroxylase did not modify the clinical or chemical manifestations. This condition resembles scurvy except for the lack of correction by ascorbic acid.

Structural defects of the fibrous proteins can also be acquired by imposing abnormal functional conditions on compounds otherwise normal in their primary structure. Such a process can occur either by adding an inhibitor of one of the enzymes, suppressing a required cofactor, or by removing an inhibitor. Scurvy has been known for a long time to result from the absence of vitamin C. Reduced hydroxylation of prolyl- and lysyl- residues in collagen, basement membranes polypeptides, and elastin probably leads to the retardation of growth, depressed healing, and rupture of blood capillary walls. Copper deficiency experimentally induced in pigs results in a depressed activity of lysyl oxidase and the lack of formation of crosslink precursors. Aneurysms and blood vessel rupture can result from this alteration. In some animal species, lathyrogenic compounds (aminonitrils) inhibit lysyl oxydase, preventing also the formation of crosslink precursors. In lathyrism, the collagen framework is most altered, leading to fragility of skin and tendon, bone deformities, and cardiovascular pathology; changes most striking in growing animals. Elastin is also altered, but the clinical expression of its defect is less obvious.

The two sugar transferases seem to be under control of insulin or products of the intermediary metabolism regulated by this hormone. Their activity is increased in experimental diabetes (Spiro & Spiro, 1971). In the human, such defective control might be responsible for alterations in the basement membranes of various blood vessels, and perhaps the dermo-epidermal junction.

An imbalance between the process of synthesis and degradation of any structural protein, of otherwise normal composition, leads to a control defect. This can be genetically determined or acquired.

The Marfan syndrome is an inherited disorder of several, but not all, connective tissues. The syndrome is characterized by a fragility of the large blood vessels and the fibres suspending the lens, abnormal development of the skeleton and tendons, fascia and ligaments. Chemical alterations have not been observed in collagen or elastin. In the aorta, the connective tissue fibres (elastic and collagen) are disrupted by the accumulation of proteoglycans (Bolande, 1963). The increased rate of collagen turnover, evidenced by the increased urinary excretion of peptide-bound hydroxyproline, might be indirectly related to the presence, in excess, of the polysaccharides.

In dystrophic epidermolysis bullosa, the formation of friction blisters correlates with an increased activity of collagenase (Eisen, 1969). In this recessive disease, a defective gene might be responsible for an abnormal control of this enzyme activity, either by local increased synthesis, enhanced activation, or reduced inhibition.

Progeria and associated syndromes are genetic and often inherited. Most connective tissues are affected and their modifications are similar to those observed in senile skin, an acquired control defect. Imbalance in metabolism, synthesis being proportionally more reduced than degradation, might be responsible for the observed modifications. The apparent increase in elastic fibres might be related to a more rapid removal of collagen than elastin. Prolonged corticosteroid therapy produces a similar defect. Osteoporosis simultaneously observed in such patients might be related to a similar process.

Although keloids are always acquired defects related to healing, some genetic predispositions are known to exist. The connective tissue plays a homeostatic function in filling any loss of tissue. Normally, the granulation tissue undergoes a progressive remodelling to establish a fibrous scar displaying little metabolic activity. This physiological behaviour seems to be conditioned by the overlying epidermis. In keloids, the progressive reduction of metabolic activity does not occur as in normal scar; the continuing remodelling might be related to an abnormal control of the fibroblast activity, perhaps by a defective messenger of epidermal origin.

Scleroderma, scleromyxoedema, and pretibial myxoedema are also acquired defects resulting from an imbalance in different metabolic processes affecting the connective tissues in several locations. The occurrence of pathological immunoglobulin in these three conditions suggests a possible interference of antibodies with either enzymes or signals involved in the control of metabolism. Soft connective tissue calcification, an abnormal chemical property of the dermis, might also be related to a similar type of mechanism. As the mineral deposition in soft connective tissues occurs within glycoprotein matrix under conditions similar to those prevalent in bone (Nusgens, Chantraine & Lapiere, 1972), it is tempting to assume that a trigger mechanism induces the production by dermal fibroblasts of a normally repressed type of structural glycoprotein, similar to that produced by osteoblasts. This would represent an example of an acquired control defect of structural glycoproteins as observed in hyperparathyroidism, renal failure, and tumoral calcinosis.

There are so many molecular processes, known or still hypothetical, responsible for the formation, organization, and maintenance of the connective tissue that all the pathology of this widely distributed structure can be explained in terms of molecular pathology. Even though a variety of abnormal chemical processes can lead to defective fibres, most of the clinical expression of this pathology is related to their abnormal physical properties. Correct mechanical resistance of the collagen fibres probably depends on all, or most, of the chemical properties of the constituent polypeptides of the structural proteins.

When an event leading to a malfunction of an enzyme, such as the presence of an inhibitor or the lack of a cofactor, is applied to the organism, the whole connective tissue is altered, even if some detail in the primary structure of the molecule differs from tissue to tissue. The basic processes and the enzymes involved are either identical, or so closely related that all the molecules synthesized under the abnormal condition will be modified. In such a situation the extent of the defect and its mechanical expression will mainly depend on the rate of remodelling of the tissue. In terms of acuteness of such defects, skin and bone, the two fastest remodelling tissues, will be most strikingly altered, while tendon, fascia, cornea, and large blood vessels, particularly in the adult, will suffer the least.

Alterations of the genetic material leading to the synthesis of polypeptides abnormal in their primary structure might exist, but have not as yet been demonstrated. In this situation, all the tissues using the same set of cistrons would display altered properties, while other connective tissue using different cistrons would be unaffected. Such alterations will be more difficult to detect in clinical investigations, since more sophisticated chemical analyses using larger amounts of tissue would be required.

When an enzyme involved in the modelling of the polypeptide chain is deficient, as in the heritable disorders, dermatosparaxis and lysyl-protocollagen hydroxylase deficiency, the situation looks more complex and is not yet completely understood. In both diseases, skin is most affected, while other connective tissues using similar cistrons for collagen synthesis are less modified. Although the inherited defects are recessive, and most probably only the homozygous state would allow their expression, even in skin where they are most pronounced, some reaction product(s) of the missing enzyme can be detected. This might correspond to the presence of a mutated enzyme of low activity, a hypothesis presently under investigation in dermatosparaxis.

In genetically determined diseases, the defect can be demonstrated in culture of fibroblasts collected from the patient and his progenitors. This situation makes it possible to envisage a future in which enzyme, or even gene, therapy will be able to correct such deficiencies. In this respect, dermatology is a favoured discipline, because of the easy access to the site of the anomaly and the striking expression of the defect in the dermis. In the prospect of such a future, animal models are most valuable. Dermatosparaxis is indeed known in the calf, but possible examples of such a disease might exist in the human.

Human and animal genetic diseases also provide the biologist with wonderful tools for investigating the structure–function relationship of the structural components. Much progress in molecular biology has occurred through the observation of mutants in micro-organisms. Molecular biology applied to organisms composed of many cells and a connective tissue is still in its infancy as compared to molecular biology of unicellular organisms, and even in this latter case much still has to be understood in the regulation of the biochemical events.

Service de Dermatologie
Hôpital de Bavière
Liege, Belgium.

CH. M. LAPIERE

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Society proceedings

Irish Dermatological Society

30 September 1972, Belfast

Chairman: DR DAVID MITCHELL

Acquired Hypomelanosis in a Guyanese DR M.A.T.KELLY

A woman aged 54

History. Her nationality is Guyanese and she was born of Indian parents. Following a confinement 20 years ago she commenced to lose her skin pigmentation. The depigmentation commenced on her forehead and then became generalized until she was virtually a white-skinned person. There is no family history of this or any other skin disorder. She had not been taking any drugs which might have precipitated the condition. The presenting problem now is that she has commenced to repigment and has a piebald appearance, which is naturally causing her great distress (Fig. 1). One felt it was important to exclude initially the possibility of a basic autoimmune disease. *Investigations*. Hb 15·5 g/100 ml; WBC 5700/mm^3; normal differential; ESR 4 mm/h. No LE cells seen. Glucose tolerance test normal. PBI 5·2 μg/100 ml. Serum cortisol 15·5 μg/100 ml at 8 a.m., 8·0 μg/100 ml at 12 p.m. Urinary 17-oxosteroids 6·1 mg/24 h (normal 5–17); urinary 17-oxogenicsteroids 6·7 mg/24 h (normal 5–18). Antinuclear factor, anti-thyroglobulin precipitation test, and thyroid antibody Latex test negative Thyroid complement fixation test positive to a titre of 1/32; Thyopac T3 uptake 107·9; RA latex test negative. No antibodies to intrinsic factor detected. Gastric parietal cell antibodies positive. Rose-Waaler test negative. ECG: sinus rhythm; record within normal limits.

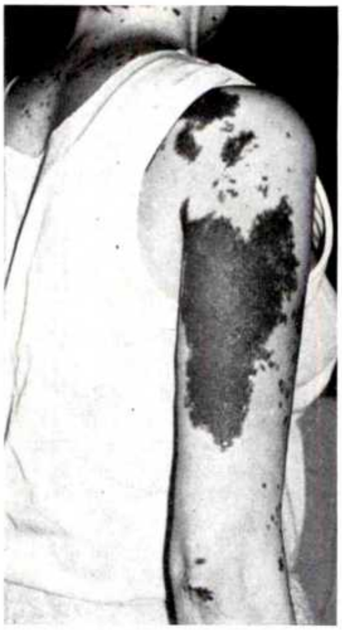

FIGURE 1.

Comment and Course. It was felt that an effort should be made to decolorize the repigmented sites in this patient. Oliver, Schwartz & Warren (1939) were the first to report areas of depigmentation developing after contact with rubber containing the anti-oxidant monobenzylether of hydroquinone. Zakon & Goldberg (1951) reported nine cases of leukoderma in white workers who came in contact with dust and debris containing this anti-oxidant. Spencer (1962) pointed out that monobenzylether of hydroquinone was equally effective as a depigmenting agent in concentrations of 1, 4, 7, and 10% and that inflammatory reactions generally precede depigmentation.

The patient was given a lotion of 5% monobenylether of hydroquinone in mid-October 1972 to apply twice daily. She was reviewed following 6 weeks of this treatment. There was no improvement. Following a suggestion by Fitzpatrick (1972) treatment has been commenced in November with 20% monobenzylether of hydroquinone.

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Incontinentia pigmentia DR DESMOND BURROWS

A girl aged 8 weeks

History. She was seen when 5 days old with a diffuse red generalized erythematous rash. On the ankles, feet, toes, and the backs of the hands there were blisters. Initially, these were discrete and scattered

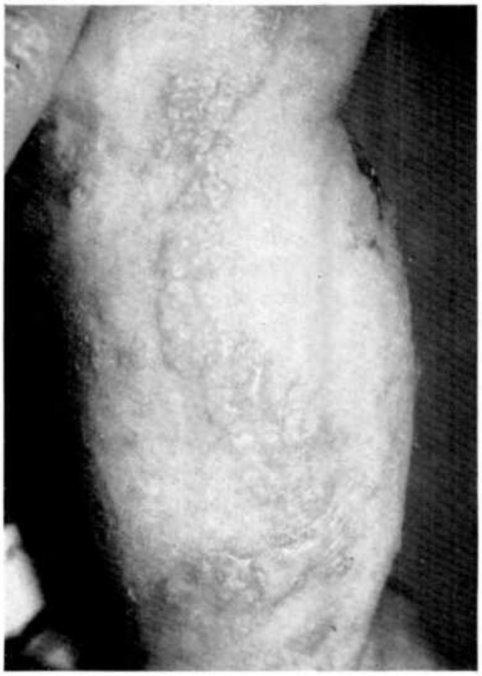

FIGURE 1. Incontinentia pigmenti, aged 10 days.

in the areas described, but within a few days the erythema subsided and the blisters became linear, particularly marked on the lower limbs, but also on the arms and buttocks (Fig. 1). New blisters appeared for a further 3 weeks and then gradually disappeared, the areas of blistering developing into linear infiltrated lesions about 2 mm wide.

Family history. This child had a normal delivery at 40 weeks gestation. Both mother's and father's blood groups are O + ve. There is no history of a child having a skin rash on either side of the family, nor is there any note of this in the GP's records. This was the mother's fourth pregnancy. The first was an aborted male child—21 weeks. The second was a hydrocephalic female child—32 weeks. The third was an aborted male child—22 weeks.

Correspondence

IN VITRO LIPID SYNTHESIS IN RAT SKIN

SIR, We read with considerable interest in the December issue the results of Dr Prottey and his colleagues' comprehensive study of lipid synthesis *in vitro* in rat skin. In 1970 we performed a series of experiments on the lipid synthesizing ability of the various components of rat skin, the results of which were hitherto unpublished, but which culminated in our work on the isolated human sebaceous gland (Summerly & Woodbury, 1971). We are now prompted to publish these results in view of the strikingly similar findings in those experiments where the techniques employed by Prottey, Hartop & Ferguson (1972) and ourselves are comparable.

In our 'component' experiments 10 mm diameter slices of rat dorsum epidermis, with a small quantity of papillary dermis attached, were removed by an electrokeratotome. Chemical cleavage at the epidermo-dermal junction was effected by means of 0·25% trypsin in buffered saline at 37°C for 15–20 min (in histological sections the PAS staining epidermal basement membrane was evident in the dermal specimens). The epidermal discs were then *either* immediately peeled from the underlying papillary dermis, rinsed in saline, the trypsin neutralized with horse serum, and cultured in the presence of 1 – ^{14}C acetate in Krebs–Ringer bicarbonate, with added glucose, for 4 h; *or* peeled from the dermis, rinsed in saline, the trypsin neutralized, and then wrapped around the papillary dermis, which had been rolled into a ball, for the period of culture; *or* rinsed in saline, the trypsin neutralized, and left in contact with the dermis, the mechanical separation being deferred until after the culture period. Other epidermal discs were cultured without preliminary enzyme treatment, removal of the papillary dermis being effected *after* culture by 0·25% trypsin at 0°C for 20 h, during which period some lipid synthesis would continue from the ^{14}C acetate which had already diffused into the tissue from the culture medium, before the effects of cooling and the enzyme were fully established. Whichever of these four procedures was adopted the pattern of incorporation into the epidermis was essentially the same. From these experiments we have calculated that actual separation of the epidermis from the papillary dermis following trypsin digest prior to the culture (irrespective of whether the two components are re-approximated for the culture) impairs the lipid synthesizing ability of the epidermis by as much as 58% (range 40–58%), as compared with epidermal performance after chemical cleavage by trypsin *in situ* without mechanical separation.

As the isolated epidermal discs are thus greatly modified by the enzyme digestion the incorporation during the ensuing 4-h culture must be a reflection of the unaltered cells; an almost identical situation obtains in the isolated epidermal cell preparations of Prottey and his co-workers. We are, therefore, able to submit a direct comparison; the similarities are obvious (Table 1). Comparable patterns of incorporation were also obtained in the dermal 'residue', despite different periods of culture (Table 1).

The difficulties inherent in the design of 'component' experiments largely arise from the adverse (unphysiological) effects that the various methods of separation must inevitably have on the subsequent performances in culture of the skin components. Isolation of the components by means of 1 M calcium chloride ($2\frac{1}{2}$ h at room temperature) resulted in death of the tissues, and so proved unsuitable. Stretch methods of epidermal separation are inappropriate to such experiments, as the anatomical relationship between a portion of epidermis and its own dermis is lost. Heat separation in a water bath at 50°C for 3 min (after Frienkel & Aso, 1969) did not produce cleavage consistently at the epidermo-dermal junction. We agree with Prottey *et al.* (1972) that tryptic digestion for 15–20 min at 37°C will not separate rat epidermis from the underlying dermis if full thickness skin is used; a keratotome shaving of epidermis with some papillary dermis (separated with trypsin in 15 min at 37°C prior to culture) and its performance contrasted with the dermal 'residue' is one technique—albeit somewhat artificial—we are forced to adopt.

As we demonstrated that the pattern of lipid synthesis in the epidermis *in vitro* was constant, irrespective of whether separation from the papillary dermis with trypsin was effected before or after culture, all our subsequent experiments on rat skin have been designed to contrast the distribution of the in-

TABLE 1. Pattern of incorporation of ^{14}C acetate into the lipids of rat epidermis, epidermal cells and dermal 'residue' *in vitro* (expressed as % of total incorporation present in each class)

Lipid class	Epidermal disc (4 h) Summerly & Woodbury	Epidermal cells (4 h) Prottey *et al.*, 1972	Dermal 'residue' (4 h) Summerly & Woodbury	Dermal 'residue' (24 h) Prottey *et al.*, 1972
Phospholipids	51·4	50·8	16·9	12·7
Free sterols	14·6	21·8	8·1	9·8
Free fatty acids	16·4	12·5	15·7	3·5
Triglycerides	13·3	14·0	19·7	23·3
Wax esters/ sterol esters Squalene	4·4	—	39·6	43·3
		0·9		0·3
No. of observations	19		6	

TABLE 2. Pattern of incorporation of ^{14}C acetate into the lipids of rat skin (epidermis and whole dermis); separated with trypsin at 0°C in 20 h, after 4 h culture *in vitro*

Lipid class	Epidermal disc	Full-thickness dermis
Phospholipids	47·6	24·8
Free sterols	18·2	5·8
Free fatty acids	8·7	7·8
Triglycerides	11·4	18·4
Wax esters/ sterol esters	9·7	37·4
Squalene	1·4	5·7
No. of observations	56	67

corporated ^{14}C acetate between the epidermis and its underlying dermis (papillary and deep), a clean separation being consistently produced by trypsin 0·25% at 0°C for 20 h *after* culture (for full details of technique see Summerly, Woodbury & Yardley, 1972). These patterns appear in Table 2 and, allowing for different culture periods and a two solvent system of TLC, which we have since adopted, approximate to those seen in our 'component' experiments.

ROY SUMMERLY
SHEILA WOODBURY

MRC Unit on the
Experimental Pathology
of the Skin
The Medical School
The University
Birmingham 15

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THE TREATMENT OF PLANTAR WARTS WITH 5-FLUOROURACIL

SIR, In December 1970 I reported in a letter to this Journal that we were undertaking comparative treatment trials to find out whether 5-fluorouracil (5-FU) would prove significantly successful in the treatment of the mosaic type of plantar warts. The trials were carried out according to the design used by us previously, and found to give valid results (Bunney *et al.*, 1971).

It became evident during preliminary pilot studies that patients found the 5% 5-fluorouracil ointment, recommended by Hursthouse (1970), difficult to keep in place under an occlusive plaster. Solutions of 5-FU in penetrating solvents were therefore used and allocated randomly with other materials under test. The cure rate at 12 weeks was assessed, and the following results relevant to 5-FU were obtained:

Mosaic trial details	Material tested	No. of patients treated	Cure rate %
103 patients 3 materials	2% 5-FU in propylene glycol	28	46·5
59 patients 3 materials	5% 5-FU in dimethyl sulphoxide	15	53·3
	Salicylic acid paint*	16	50·0

* Salicylic acid, 1 part; lactic acid, 1 part; flexible collodion, 4 parts.

The cure rate for the Salicylic Acid Paint was identical with that obtained in previous trials carried out on the same design. 2% 5-FU in propylene glycol was found by Honeycutt, Dillaka & Jansen (1967) to have a potency equal to 5% 5-FU ointment. It proved impossible to obtain a solution of 5-FU at the 5% level in propylene glycol, therefore the different vehicle was used. Even at this higher concentration the cure rate for 5-FU is not significantly greater than that obtained with a simple keratolytic agent. We are, therefore, of the opinion that its use is unjustified in the treatment of warts, even those of the resistant mosaic variety.

MARY H. BUNNEY

Wart Clinic, Department of Dermatology
The Royal Infirmary, Edinburgh EH3 9YW

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DISTRIBUTION OF CONSULTANTS IN ENGLAND

SIR, I have recently been looking into the distribution of consultants in dermatology throughout England, using the latest figures available. I realize that during the last 12 months several new consultant posts have been established and filled, but even so, there are still striking differences between different parts of the country. I realize also that there are great variations in the rate of work of individual consultants, and the degree of supporting help also differs, but with the increasing complexity of disease processes one is drawn to the inevitable conclusion that several areas are understaffed. Requests for increasing the consultant establishment are commonly made in the first instance by the Medical Staff of the Hospital Group or Groups concerned to their Regional Board. If the Regional Board is of the opinion that the request is reasonable, and can be supported, this is then forwarded for consideration to the Department of Health

Region	Consultant sessions (WTE)	Population in millions	Consultant/ population ratio
Newcastle	10·9	3·1	1/282,000
Leeds	10·0	3·2	1/320,000
Sheffield	12·4	4·6	1/370,000
Cambridge	4·9	1·8	1/360,000
Oxford	9·8	2·0	1/204,000
Bristol	8·6	3·1	1/360,000
Birmingham	14·9	5·2	1/347,000
Manchester	7·2	4·6	1/639,000
Liverpool	3·6	2·3	1/640,000
Wessex	5·0	2·0	1/400,000
N.W. Metropolitan	18·3	4·2	1/230,000
N.E. Metropolitan	10·6	3·4	1/320,000
S.E. Metropolitan	11·7	3·6	1/307,000
S.W. Metropolitan	9·0	3·3	1/366,000

and Social Security who give the final approval for increasing the establishment. The Regional Board may then implement this if they have the money available within their budget.

Your readers may be interested in the figures available to me affecting consultant establishment expressed as whole time equivalents (WTE); the regions include the teaching hospitals. (See p. 97.)

H. R. VICKERS

Blackhall Farm
Charlbury Road
Oxford OX2 6UX

BETEL CHEWERS' PERLECHE

SIR, Perleche is a term derived from the French, and means to lick thoroughly. It is generally applied to inflammatory processes occurring at the angles of the mouth.

In India, cracks and fissures of varying degrees of severity are frequently seen at the angles of the mouth in chain 'betal chewers'. This letter reports eleven patients with this condition seen in a period of 9 months.

Banaras is situated in the eastern part of India, where betel chewing is a common practice amongst males and females. There are some who take it only after food, but many chew it perpetually. Two or four 'betels' are put in the mouth, along with tobacco mixed with lime (calcium oxide). This mixture is usually kept behind the lower lip.

'Paan', as betel is called locally, consists of betel leaf (from the pepper plant, *Piper betle*) coated with a thick paste of lime and 'Katha', or catechu, a mixture of astringent substances derived from bark of *Acacia catechu*. These substances, including pieces of betel nut are put on the leaf and rolled.

Continuous chewing keeps the mouth full of saliva, which trickles to the angles of the mouth which are constantly moist. The continuous friction of moist opposing surfaces at the angles of mouth causes maceration, erosion and cracks.

The lesions were characterized by a sodden area at the angles of the mouth, chiefly on the cutaneous side of the muco-cutaneous junction. The lesions were bilaterally symmetrical in five of eleven patients. In the more severe cases the sodden area included deep painful fissures.

'Chain' betel chewing was a constant feature in the nine men and two women. The duration of betel chewing had ranged from 3 to 12 years. Seven of these patients sought treatment at the clinic for the cracks and painful lesions. Three of these seven had erosions of the buccal mucosa, and experienced burning and pain on taking hot and irritating foods. They could no longer chew betel. Four patients were noticed by the author when they have come to him for treatment of some other condition.

Culture for Candida species was done in only five patients, and only one yielded *Candida albicans*. In no patient was there evidence of vitamin deficiency; their general condition was good. Two of these subjects were, nonetheless, given heavy doses of vitamin B complex, but without a favourable response. *C. albicans* found in the one subject was perhaps a secondary invader.

The patients were advised to stop the use of betel or tobacco with lime. Seven patients could be followed-up. Erosions and cracks disappeared in 2–6 weeks.

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The author is thankful to Dr K.N. Udupa, Senior Medical Superintendant, S.S. Hospital, Institute of Medical Sciences, BHU, for permission to publish this report.

GURMOHAN SINGH

Institute of Medical Science
Banaras Hindu University
Varanasi-5, India

PSORIASIS AND OBESITY

SIR, The management of psoriasis is influenced *inter alia* by personal experience and beliefs. One belief of mine was that chronic generalized psoriasis in the obese subject could be benefited, occasionally dramatically, by strict dieting to produce a significant loss of weight. Of course the advantages of weight-loss to the intertriginuous type of psoriasis are obvious.

When asked recently by a former pupil for documentation of the value of a reducing diet for psoriasis, I could not find a report of a controlled series of cases so treated. Nor was there a record of any statistical association between the severity of psoriasis and obesity.

I would be grateful for any relevant information from your readers.

LOUIS FORMAN

22 Harley House
Marylebone Road
London, N.W.1.

HYPOPIGMENTED LESIONS IN LEPROSY

SIR, It was enlightening to read the article by Job, Nayer & Narayanun (1972), published in the columns of your Journal. The authors in their preliminary report have incriminated atrophy and decreased activity of melanocytes, coupled with the reduction in their number, as the probable cause of hypopigmentation in indeterminate leprosy. Earlier Prabnakaran, Harris & Kircheimer (1971) reported that *Mycobacterium leprae* possesses an enzyme, adiphenol oxidase, which oxidizes DOPA, and postulated that utilization of DOPA by the acid-fast bacilli results in its depletion, thus interfering with the process of melanogenesis in leprous lesions; hence the hypopigmentation. If the observation made by Praphakaran *et al.* (1971) holds good, then the lesions of lepromatous leprosy, which harbour enormous numbers of acid-fast bacilli, should be hypopigmented, but they are not. Thus, the pathogenesis of hypopigmentation is still uncertain.

Chatterjee (1955) had suggested that reduction in the cutaneous vascular supply leads to atrophic changes in the melanocytes, which in turn reduces pigmentation in the affected regions. His observation seems plausible in the light of our clinical experience. The return of pigment to the hypopigmented lesions is striking after diamino-diphenyl-sulphone (DDS) therapy. The repigmentation, although slow, definitely occurs. It is possibly because DDS helps to decrease the cellular infiltrate, thus resulting in partial or complete restoration of nerve as well as vascular supply. Consequently, there might be an increase in the number and activity of melanocytes. These clinical observations require laboratory investigation.

Future investigations should, therefore, attempt to assess the status of cutaneous vascular supply and nerve changes, and the number and activity of melanocytes, before, during and after institution of DDS therapy in hypopigmented lesions of indeterminate, maculoanaesthetic, and tuberculoid types of leprosy. The study of these variables may assist in explaining the pathogenesis of hypopigmentation in leprous lesions.

V. N. SEHGAL

Department of Venereology and
Dermatology
Goa Medical College
Panaji, Goa, India

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Book Reviews

A Guide to Drug Eruptions. W.BRUINSMA (1973) Amsterdam: Excerpta Medica. Pp. 102. Price £4.09.

Several books on drug reactions have been published in the past few years, some of them excellent, but there was room for a concise and compact work, written specifically for the dermatologist, and preferably by a dermatologist. There can surely be no one better qualified for this task than Dr Bruinsma, who is Director of Public Health for Drugs in the Netherlands Ministry of Public Health, and is also a practising dermatologist at West Friesland and Hoorn. Dr Bruinsma's attractively designed volume presents a convenient short practical guide to drug eruptions, which compresses into 100 pages a large amount of essential, well documented, and up-to-date information.

Short introductory chapters discuss the classification of drug reactions and the diagnostic procedures available. The next eighteen chapters describe the clinical syndromes caused by drugs and list the drugs most commonly responsible. After a useful list of references there follows an index of drugs with the reactions each most commonly causes. The book ends with a detailed index of cutaneous signs or symptoms, guiding the reader to the main text or to the references.

As a whole the book is well written in clear and correct English and a few lapses, such as inducement where induction was intended, are of little importance, as the meaning is not obscured. Unfortunately there are others which are less easy to understand; what, for example, is 'a hirsute type of alopecia'?

As new drugs are introduced in such numbers, guides like this are an invaluable aid to any dermatologist in practice. Dr Bruinsma is to be congratulated on achieving that degree of clarity and simplicity which are the fruits of scholarship and of tedious labour. His book deserves a wide circulation, and it is to be hoped that its success will encourage him to provide revised editions at regular intervals. Perhaps he will ask an English colleague to read his typescript.

ARTHUR ROOK

Common Problems in Office Practice. Current Methods of Diagnosis and Treatment. ROBERT B.TAYLOR (1972) Hagerstown, New York, Evanston, San Francisco, London: Harper and Row. Pp. 402. Price £5.00.

Education for general practice and the continuing education of the general practitioner are rightly of growing importance. One difficulty is in defining the content of general practice and in isolating those qualities which give general practice its distinctiveness, as opposed to a mere assemblage of specialist subjects.

Dr Robert Taylor is the American equivalent of the British general practitioner. He has been in practice in upstate New York for 10 years or so. In his book, 'Common Problems in Office Practice', he has attempted to cover the field of general practice by writing a book divided into a chapter dealing with 'general office management' and going on to 'areas of common office problems': Internal Medicine, Dermatology, Infections Diseases, Paediatrics, Urology, Gynaecology, Surgery, Orthopaedics, Psychiatry, and Drug Abuse. Each of these is broken down into sections dealing with specific ailments, arranged alphabetically for quick reference. He hopes that the book 'will save the new practitioner much trial and error learning, and will offer up-to-date advice to the established physician'.

Dr Taylor writes in a direct readable style with none of the turgidity sometimes associated with texts from North America. The simplicity sometimes goes too far. For example there is a section entitled —'Flu'—'properly called influenza, is an epidemic disease caused by several strains of viruses'.

The section on practice organization ('general office management') is surprisingly relevant to practice in the U.K.; medical records, ancillary help, appointment planning, how to handle the patient load, and preventive medicine are all of topical importance.

Each clinical section gives an account of the manifestations and management of the condition, interspersed with historical snippets. Dr Taylor is at his best in his description of management. Detailed advice is given not only on medication, but in the manner of its application; for instance, the way to put drops into the eyes of unwilling children. He discusses the effect of the illness on the life of the patient and what must be altered to effect optimum chances of cure or alleviation.

He condemns the practice of diagnosing and prescribing over the telephone and in other ways shows the soundness of his own clinical methods.

He devotes thirty-eight pages to dermatology; the fourth longest chapter. He includes sections on the more common conditions, although Poison Ivy dermatitis might not gain a place in this country. There are no illustrations, a serious limitation in any book which deals with diagnosis in dermatology. The space given to 'manifestations' is variable, and to some extent arbitrary. For instance, he gives one page to manifestations of herpes zoster, but only a quarter page to manifestations (despite nearly two pages to treatment) of psoriasis. Recommendations for treatment are mostly sensible. He is cautious about the use of systemic corticosteroids, but is prepared to prescribe short courses for severe seborrhoeic dermatitis, urticaria, selected cases of severe psoriasis (he warns of rebound) and severe cases of contact dermatitis. In this country it is unwise for a general practitioner to prescribe systemic corticosteroids for any skin condition without prior consultation with a dermatologist. This needs to be clearly taught.

Each chapter has references to reviews and original articles; more suitable for this book would be references for further reading, rather than original papers.

This book is unlikely to be of value to the experienced general practitioner. He needs reference books giving full details of uncommon diseases or unusual manifestations of common conditions. Although it is sometimes useful to have a full account of established methods of management, his main need is a critical evaluation of new drugs and treatments.

The general practitioner in training will not find enough detail to satisfy his needs for clinical diagnosis, but the treatment sections should be useful to him.

The book may find a place for the inexperienced doctor or locum going straight into practice without training, as a quick reference 'bench book'. It could have a real place for nurses attached to family practice. Anyone wishing to study medical care in the U.S.A. would find it interesting.

Dr Taylor will have to expand his book to make it more generally useful. He might, however, in his next edition confine himself to 'current methods of *management* in office practice.'

BERNARD REISS

Das Bild auf der menschlichen Haut. DIETRICH VON ENGELHARDT (1972) Munich, Heinz Moos. Pp. 96, illustrated.

This slim paper-backed volume presents a remarkable collection of photographs of tattoos, accompanied by a very brief text on the history of this ancient art and the motivation of those who submit to its practice. The designs are classified in a number of broad groups, such as love and hate, power and masculinity, flowers and animals, humour and obscenity, and religion. The photographs are of some sociological interest, but the list of only eleven references does not compensate for the disappointing brevity of the text.

ARTHUR ROOK

Penicillin in the Treatment of Syphilis: The Experience of Three Decades. O.IDØE, T.GUTHE AND R.R.WILLCOX (1972) Geneva: World Health Organization. (Supplement to vol. 47 of the *Bulletin of the World Health Organization.*) Pp. 68. Price £1.20. Available through H.M.Stationary Office, 49 High Holborn, London, W.C.2., or from booksellers.

Since penicillin was first used in the treatment of syphilis in the United States of America in 1943, considerable experience of its effectiveness against the early, late, and congenital forms of the disease has been gained. In this supplement to the *Bulletin of the World Health Organization*, prepared with the collaboration of an international group of investigators, the authors draw on a wide range of published material and personal experience to describe treatment methods and results over the past three decades.

Data are tabulated on 14,460 patients followed for up to 18 years after treatment—5,652 with early syphilis and 8,808 with late syphilis—in studies that satisfied the authors' criteria of completeness and comparability. Among the patients with early syphilis the failure rate varied from nil to about 1%, although the rate of retreatment after new infections was higher. The clinical and serological results in late syphilis were generally satisfactory, bearing in mind the extent of tissue damage before treatment.

Despite the intensive use of penicillin for three decades, *Treponema pallidum* has shown no sign of resistance to it. Because of its ease of application, low toxicity, and moderate cost, penicillin continues to be remarkably effective in the prevention and cure of syphilis and other treponematoses, and is the drug of choice in almost all countries.

The volume contains extensive tables and some 630 references.

Textbook of Dermatology. ARTHUR ROOK, D.S.WILKINSON AND F.J.G.EBLING (Eds.) (1972. Oxford and Edinburgh: Blackwell Scientific Publications. 2nd Edition. Pp. xliv +2118, illustrated.) Two vols. Price £32.50.

The appearance of the second edition of this book comparatively soon after the first testifies the success with which it has fulfilled a need.

The list of authors shows a few alterations, but although in the preface the editors mention that most chapters have been extensively revised and very many entirely rewritten, this is probably not on a scale which renders the first edition by any means obsolete. Although in some places the text has been much altered in view of recent advances, there are others which have been reworded without apparent alteration in meaning, and some where the meaning has been revised but without mention of any fresh factual evidence on which this is based. This rewording or rethinking may show up deficiencies in the original version but does not always appear to be an improvement.

Tables of references mostly show the addition of some recent publications, but these may or may not be accompanied by an alteration in the text; and it is possible to find places where a fresh reference is not correctly attached to the revised text.

Contrasts and comparisons between the two editions could be indefinitely multiplied. On balance it is reasonable to propose that the second edition is an improvement on the first, but changes do not suggest that advances in dermatology in the past 5 years have made the first edition hopelessly out of date.

The general format is unchanged. The second edition has been enlarged by 10% and the price has unfortunately increased by 50%. But these two volumes are essential to the dermatologist; the trained dermatologist will need them for frequent reference, while for the trainee they should be the sheet-anchor of systematic study.

F.RAY BETTLEY

Actualités de Dermopharmacologie. VIe Semaine Internationale de Dermopharmacologie. Organized by the European Centre for Dermatopharmacology, Lyon. Pp. 199.

This is a rather curious mixture of papers given at a conference in Lyon, January 1972. The majority are concerned with the biochemistry and biophysics of aging skin, but there is a frothy top to this heavy brew, consisting of papers on cosmetic surgery of the aging scalp and face and the like.

There is first a general account of the biology of aging which raises some interesting questions, but answers none: then A.Huc and Ph.Comte get down to things and discuss (very well) the application of physico-chemical methods in the study of aging collagen, especially their use of X-ray diffraction and the effect of penicillamine and the lathyrogenic agent β-amino-propronitrile on collagen cross-linkages. Next, P.Divry, A.Huc, Ph.Comte and J.Cotte describe two cases of progeria; brothers aged 5½ and 3½ years. They noted abnormally low levels of hydroxyproline excretion, but were unable to confirm previous reports of increased mitochondrial respiration, though the permeability of these organelles (isolated from muscle biopsies) was abnormally great. Growth hormone levels were normally stimulated by arginine, although, as in other cases, there was a less than normal response to insulin. Infra-red spectroscopy of collagen extracts revealed only slight deviations from the normal in the amide band, but X-ray diffraction showed a clear similarity to the pattern observed in old people's skin, as did measurements of the temperature of retraction of collagen. Amino-acid analysis of collagen indicated levels of hydroxylysine and hydroxyproline well below controls. They discuss also the life span of fibroblast cultures; reported previously as being diminished, but add no data of their own. Nevertheless, the authors present an interesting laboratory view of this caricature of old age.

M.Lapras presents a review and his own experience of the use of sulphadiagine in the retardation, and even reversal, of the skin changes which usually accompany aging. He cites earlier work by Hackermann, Kaemerer and others, but gives no references; the claims made in this paper seem to me to be extraordinary, but are not here at any rate backed up by the sort of data one would wish to see for such startling assertions. One paper prefaces quite a nice account of hair grafting procedure for 'l'alopécie séborrhéique androdistrophique' with a discussion of the 'Emden–Mayerhoff–Kreb cycle' (*sic*) and cyclic AMP in relation to the hair follicle—a marriage of the theoretical and the practical which we must surely all applaud. Surely Professor D.Colomb cannot be the first to describe the stellate scars so commonly seen on the dorsal aspect of old people's forearm and hands? Perhaps the gallant French have hitherto studiously avoided remarking on this external manifestation of collagenous decay. However, Colomb does show some very good clinical illustrations; the histological photographs are less satisfactory.

H.Tronnier of Tübingen describes the elastic properties of skin as a function of age, using linear stretch, torsion and suction methods, while F.A.J.Thiele of Nijmegen describes his work on the water content of stratum corneum and the effect of atmospheric humidity, and compares the results with those obtained with artificial membranes. This is a well-presented and interesting paper which is difficult to summarize, and should be seen in its entirety.

M.Schmitt discusses measurement of skin temperature using both contact methods and infra-red telemetry, but few experimental results are documented.

W.M.Chang describes the effect of soap and other agents on skin as seen by scanning electron microscopy of excised fragments, and relates the changes to water-binding capacity and stress–strain curves.

In summary, this bouillabaisse of a book has some fascinating and occasionally startling ingredients; to stodgy British tastes it may be a little exotic, but perhaps we shall become accustomed to taking things with a pinch of garlic.

STANLEY COMAISH

The Lymphatics: Disease, Lymphography and Surgery. J.B.KINMONTH (1972) London: Arnold. Pp. 420. Price £12.50.

Professor Kinmonth has been one of the chief pioneers in the study of lymphatic disorders, and especially in developing the techniques of lymphography, over the past 20 years. He has an unrivalled experience of investigating and treating such patients. This book is largely a careful account of his experiences at St Thomas', and also those of the team of St Bartholomew's, together amounting to over 2000 patients. The first section explains in detail the techniques, uses and hazards of visual and X-ray lymphangiography, and the normal lymphatic anatomy, not only of the limbs but also of most of the rest of body. There follows an account of the clinical types of lymphoedema, including those involving the head, with an attempt to correlate these with the various X-ray and visual lymphographic appearances with prognosis and with the most suitable surgical or conservative treatment, both of which are discussed in detail. It is apparent that careful assessment allows more help to be given to these patients than is often attempted. Professor Kinmonth is also an advocate of surgical removal of 'lymphangioma circumscriptum' (a name he rightly dislikes). This he often does in stages, if necessary taking such precautions as visualization of the lymphatic vessels at the time of operation to avoid damage to them.

The book is beautifully illustrated with black and white and colour photographs, and clearly contains a great deal that is of importance to dermatologists.

R.H.CHAMPION

Methods in Microcirculation Studies. T.J.RYAN, B.JOLLES AND G.HOLTI (Eds.) (1972) London; H.K.LEWIS. Pp. 128, 44 illustrations, including one in colour. Price £3.60.

This small book is based on a symposium of the British Microcirculation Society held in 1971. This flourishing society draws together workers from many fields who share an interest in the behaviour of blood and small blood vessels, and who might never normally make contact with each other. Likewise, this book brings together in a series of eighteen papers an account of various techniques, the majority of which are primarily concerned with circulatory phenomena in the skin. Some of the papers are descriptions and evaluations of techniques, usually fairly brief, but with references to more detailed accounts; others are less concerned with techniques and give a review of some fundamental problem, e.g. inflammation. The book is not at present comprehensive, but is in loose leaf form and further additions are promised. It contains much useful and interesting material, not only for the research worker interested in methods but also for the clinician interested in the results, for example, in such rapidly expanding fields as fibrinolysis and the skin. There is a useful bibliography, both general as well as related to the particular problems; few dermatologists can be familar with the majority of these references.

R.H.CHAMPION

Skin, Heredity and Malignant Neoplasms. H.J.LYNCH (Ed.) (1972) London; Lewis. Pp. 300, illustrated. Price £9.20.

Dr Henry Lynch, the editor and the principal author of this unusual book, is professor of preventive medicine and public health at Creighton University School of Medicine, Omaha, Nebraska. Only one of the remaining sixteen authors is a dermatologist; the others include general physicians, paediatricians, geneticists, oral pathologists, a surgeon and a radiobiologist. As the editor explains in his preface, a family with xeroderma pigmentosum first brought to his attention the value of cutaneous changes in the early diagnosis or planned prevention of malignant disease in a wide variety of syndromes. He has assembled a team of authors in many specialties who are interested in making more widely known the importance of the cutaneous manifestations of such syndromes in preventive medicine—'in such a way that the practising physician could apply this knowledge to his busy clinical practice'.

An introductory chapter on the *Fundamentals of Genetics* by A.R.Kaplan is followed by a general review of *Cutaneous Clues to Internal Malignant Disease* by Harold Perry and his colleagues and an account by Riccardi and Cleaver of the latter's now well known work on DNA repair synthesis. The next ten chapters review systematically and thoroughly the hereditary syndromes which are associated with a significantly increased incidence of malignant disease. The last chapter discusses *Genetic Counselling*.

There are numerous black and white illustrations, and there are three pages in colour. The latter and many of the former are clear and informative, but some are of poor quality.

The dermatologist will find in this book relatively little that is new to him other than the approach, with its deliberate emphasis on prevention. However, these excellent reviews of rare diseases are certainly useful, and the lists of references are unusually comprehensive and international. The book deserves a place in the department's library.

ARTHUR ROOK

British Journal of Dermatology (1973) **89**, 105. Paper 215

Quarterly Review

DRUG REACTIONS XXII

Drug induced changes in pigmentation

Increased or decreased pigmentation may occur in the skin and its appendages. It may be due to many factors, including inflammation, or may accompany or follow certain skin diseases, such as lichen planus and leprosy. These pigmentary changes may be localized or generalized, and their degree is considerably dependent on racial factors.

Drugs may also cause changes in pigmentation, most commonly an increase. It may be difficult to define whether the drugs, or the conditions for which they are given, are mainly responsible for the change.

The colour of normal skin is due mainly to four pigments, namely oxyhaemoglobin, reduced haemoglobin, melanin and carotene. Several mechanisms are involved in drug induced changes in pigmentation of the skin; for example, heavy metals are deposited diffusely in the dermis bringing about a change in colour as a result of a Tyndall effect (optical changes in the refraction and scattering of incident light). Some elements, for example arsenic, cause colour changes by altering melanin deposition. Other drugs, for example dapsone, produce changes by combining with haemoglobin to form methaemoglobin. The fixed drug eruption produces localized increased pigmentation as a post-inflammatory effect by increasing melanin deposition. Many drugs produce pigmentary changes by unknown mechanisms.

ELEMENTS

A diffuse deposition of metals may occur in the skin and mucous membranes, resulting in a change of colour. This colour is partly due to the metal itself, as mentioned above, but may be due to an increase in synthesis of melanin, as in the case of hyperpigmentation due to arsenic. In the resting phase, tyrosinase activity of epidermal melanocytes is partially inhibited by the binding of enzymic copper to sulphydryl groups. Arsenic combines avidly with sulphydryl groups and may thus free copper and activate tyrosinase. On the other hand, mercury, gold and silver can displace copper from tyrosinase, inactivating the enzyme. This could account for the paradoxical bleaching effect of topically applied mercury, but not for the darkening effect produced when these metals are given systemically (Lerner & Case, 1959).

Arsenic and gold may both produce lichenoid eruptions with resulting post-inflammatory hyperpigmentation.

Inorganic arsenic

Inorganic arsenic was used in the past for many conditions, including psoriasis, and continues to be prescribed in some countries. It is still found in some 'tonics'. Occupational exposure also occurs (Birmingham *et al.*, 1965), and water supplies may be contaminated. Prolonged ingestion of inorganic arsenic may result in a diffuse macular pigmentation, most pronounced on the trunk, where it may produce the well known 'rain-drop' appearance. Other manifestations of chronic arsenical poisoning, such as keratoses, may or may not accompany the pigmentation. The possible mechanism of arsenical pigmentation has been discussed above.

Silver (Argyria)

Until late in the last century, silver nitrate was still used systemically in the treatment of epilepsy. At present silver compounds are used only locally. The continued application of silver salts to mucous membranes may result in sufficient being absorbed to cause side effects, as may silver nitrate used in the treatment of burns.

Argyria results from the deposition of silver in the dermis and in the mucous membranes. Silver accumulates in the body over many years. The generalized pigmentation is permanent but has no untoward effect on the health (Jeghers, 1944). The changes are probably first detectable in the mouth as a diffuse slate-grey pigmentation of the gingiva and oral mucosa (Dummett, 1965). The skin assumes a bluish-grey colour with accentuation on exposed areas; it is most pronounced on the face, hands, conjunctiva and finger nails. Cyanosis has on occasion been simulated, and as a result treatment has been given erroneously for supposed cardiac disease (Jeghers, 1944).

Gold (Chrysiasis)

Gold injections have regained their former popularity in the treatment of rheumatoid arthritis, and prolonged administration of gold may result in a permanent, diffuse, blue-grey pigmentation which may be limited to exposed areas. A faint purple gingival discoloration may be seen (Dummett, 1965). The colour is mainly due to the metal itself, which is deposited in the upper dermis (Jeghers, 1944).

Bismuth (Bismuthia)

In the past, bismuth was used parenterally in the treatment of syphilis and various skin diseases (Dummett, 1965). Its systemic use is now virtually obsolete. Pigmentation from the parenteral use of bismuth is rare. It is generalized, and resembles the blue-grey pigmentation of argyria, but the blue-black bismuth line on the marginal gingiva is distinctive (Dummett, 1965).

Mercury

Repeated applications of mercury-containing ointments or cosmetics may give rise to grey-brown pigmentation, limited to areas to which it is applied, with accentuation in the skin folds (Jeghers, 1944). Burge & Winkelmann (1970), using the electron microscope, demonstrated that melanin pigmentation increased secondary to the presence of the metal.

ANTIMALARIALS

Changes in the skin pigmentation occur in about 25% of patients receiving any of the antimalarials for periods of more than 3 or 4 months (Baker, 1972).

One of the best known and earliest reported changes is a generalized intense yellow discoloration of the skin which develops when high doses of mepacrine are used in the prophylaxis of malaria. Patients with delayed urinary excretion of the drug show the deepest pigmentation (Green, 1932). Mepacrine is a bright yellow crystalline powder and is able to stain all tissues, sweat and tears (Young, 1958). Staining of the sclera is slight or does not occur, and the absence of scleral staining is a useful sign in differentiating this condition from jaundice (Moller, 1966). Amodiaquine also may produce diffuse pigmentation, but less frequently. The colour varies from a yellowish-brown to grey, and is accentuated in light exposed areas. As with mepacrine, the sclera may be stained a very pale yellow (Young, 1958). The discoloration gradually fades on withdrawal of mepacrine or amodiaquine. Yellow staining of the skin appears not to occur with chloroquine (Young, 1958). Quinine may produce a generalized hyperpigmentation (Tuffanelli, Abraham & Dubois, 1963).

Localized blue-black pigmentation may affect the pretibial areas, the face, hard palate, and subungual regions. It may be produced by any of the antimalarials. Tuffanelli *et al.* (1963) observed this adverse effect in twenty-five of 300 patients receiving antimalarials for 'collagen' diseases. Chloroquine was the most frequently used drug. Pigmentation occurred on the shins in irregular patches, ranging from small areas of diffusely scattered pigment to large patches several centimeters in diameter. Early lesions resembled ecchymoses, but with continued therapy the areas coalesced and became darker. The duration of therapy varied from 4 to 70 months, and the pigmentation decreased several months after cessation of therapy. There appeared to be a lower incidence of pigmentation in negroes. Bolam & Stevenson (1962) described the transverse bands of blue-black pigmentation affecting the finger nails of patients treated with amodiaquine. They also noticed deposition of pigment at sites of previous lesions of lupus erythematosus. Tuffanelli *et al.* (1963) also observed five patients with retinopathy following antimalarial therapy, four of whom had cutaneous pigmentary disturbances before detection of the retinal damage. They therefore advocated that patients who develop such pigmentation whilst receiving antimalarials should have regular opthalmological examinations. Histological examination of the pigmented lesions in their patients showed yellowish to dark brown pigment granules intra- and extracellularly throughout the dermis, but predominantly in the deeper areas. Stains for melanin and haemosiderin were both positive.

All the antimalarials are capable of producing lichenoid eruptions (Feder, 1949; Savage, 1958), the resolution of which tends to be followed by hyperpigmentation.

It has been established that chloroquine has an affinity for melanin (Sams, 1967). Bernstein *et al.* (1963) found that the concentration of this drug in the eyes of pigmented rats paralleled the degree of melanin pigmentation. They also noticed that chloroquine persisted at high levels in the iris and choroid for at least 28 days, whereas the concentration decreased rapidly in the other tissues examined. Sams & Epstein (1965) compared chloroquine levels in albino mice and in pigmented hairless mice, which developed a dark melanotic plaque at the site of ultraviolet irradiation. The chloroquine levels in the albino mice, which developed non-melanotic plaques at irradiated sites, fell rapidly to zero within 1 week after a single intraperitoneal injection of the drug. The melanotic plaques of the pigmented mice, however, contained high levels of chloroquine for at least 3 weeks.

Bleaching of hair was first reported by Alving *et al.* (1948) in a study of the chronic toxicity of chloroquine. This phenomenon may occur after 2-3 months of therapy, and is reversible on withdrawing the drug. It involves all body hair, including vellus. Only blond, light-brown and red-haired people are affected. It is thought that the formation of phaeomelanic pigment (yellow-red) is inhibited by chloroquine, and that the formation of tyrosine melanin (black or dark-brown) is not affected (Fitzpatrick, Brunet & Kukitar, 1958).

HYDANTOIN DERIVATIVES

At least 10% of patients receiving hydantoin preparations develop pigmentation of the face resembling chloasma. Kuske & Krebs (1964) investigated thirteen such patients, ten of whom were women. Factors contributing to the occurrence of pigmentation included solar radiation and the individual ability of the skin to produce pigment. The hyperpigmentation occurred in one patient after 4-5 months and in three patients after 1-2 years' treatment. Duration of therapy in the other patients could not be determined accurately. Tests of endocrine function revealed no significant abnormality. These authors described a method, using isolated ends of *Xenopus laevis* larvae, for studying the dispersal of melanin granules, and were able to show a direct effect of hydantoin. Skin biopsies in affected individuals have shown the pigment to be melanin (Hunter & Jenkins, 1951).

CYTOSTATIC AGENTS

Busulphan

A diffuse brown pigmentation of the face, forearms, chest and abdomen during treatment with busulphan was first described by Galton (1953). This adverse reaction has been reported to occur in 5–10% of patients, particularly in non-caucasoids with a dark complexion (Moller, 1966). Less commonly Addison's disease is simulated (Kyle *et al.*, 1961; Harrold 1966; Feingold & Koss, 1969). These patients are hyperpigmented and complain of severe weakness, fatigue, anorexia, nausea and weight loss. Buccal pigmentation is uncommon and palmar creases are normal. Laboratory tests do not show hypoadrenalism. The mechanism of this reaction is uncertain. Kyle *et al.* (1961) postulated that busulphan inactivates sulphydryl groups in the skin by 'dethiolation', which removes an inhibitor of tyrosinase. Histologically, there is increased melanin in the basal layer of the epidermis and in the dermal melanophores.

Cyclophosphamide

Hyperpigmentation of the skin, accentuated in exposed areas, occurred in children with idiopathic nephrotic syndrome treated with cyclophosphamide (Salazar, Bercowsky & Lara, 1969). A 3-year-old boy with nephrotic syndrome developed brown discoloration at the base of the finger- and toe-nails after treatment with this drug. He also had a brown line on the teeth at the gingival margin (Harrison & Wood, 1972).

Bleomycin

This drug, which is used in the treatment of various reticuloses, may cause hyperpigmentation of the skin lesions (De Bast *et al.*, 1971). Excoriations also may become pigmented (*British Medical Journal*, 1972).

CHLORPROMAZINE AND RELATED PHENOTHIAZINES

Chlorpromazine is one of the most widely used drugs in psychiatry. Its prolonged use in large doses may produce progressive pigmentation, most marked in exposed areas. Related phenothiazines may cause a similar effect, but chlorpromazine is the drug most commonly implicated (Hagermark, Wennersten & Almeyda, 1971). The pigmentation is cumulative, and begins with a tan, or golden-brown colour, in the summer months (Sanatove, 1965). With progression, the exposed areas, particularly the butterfly-area of the face, the neck and hands, acquire a slate-grey or bluish appearance. Later, a purplish tint is noted, which after several years may become quite marked (Greiner & Berry, 1964). Mild pigmentation of the cornea and anterior lens may also occur (Sanatove, 1965). More serious associated abnormalities of the eye include corneal and lens opacities, and retinopathy (Greiner & Berry, 1964).

The mechanism of this unusual pigmentation is uncertain, although it has been extensively investigated. It is well known that chlorpromazine may cause a photodermatitis in sensitized patients, but the pigmentation is never preceded by an inflammatory reaction. The accumulation of melanin, or melanin plus chlorpromazine or its metabolites, is thought to cause the purple colour (Sanatove, 1965). Post-mortem studies in subjects who have received large doses of chlorpromazine have shown extensive deposits of melanin-like material throughout the reticulo-endothelial system, and in the parencyhmal cells of internal organs (Greiner & Nicholson, 1964). Using the skin-window technique, Sanatove (1965) demonstrated transport of pigment in inflammatory cells to the blood stream. He postulated that this may be the mechanism of production of generalized melanosis. Histological examination of pigmented skin shows deposition of a golden-brown, finely granular pigment in cells situated mainly around capillaries in the superficial layers of the dermis. This pigment has the staining properties of melanin. The blue or slate-grey colour may thus be the result of a Tyndall phenomenon (Sanatove, 1965). Blois (1965) administered radioactive labelled chlorpromazine to mice intraperitoneally. He demonstrated that the drug localized in tissues containing melanin. The rate of disappearance of the radioactivity from non-pigmented tissues was markedly higher than from pigmented tissues. Electron microscopic studies, however, suggest that the skin discoloration is not due to the melanin, but rather to a drug metabolite. Perry *et al.* (1964) suggested that hydroxychlorpromazine, or a metabolite of this compound, is converted to a purple compound on exposure to ultraviolet light.

Chlorpromazine pigmentation has become a serious problem in mental hospitals. When the drug is withdrawn, the pigmentation fades very slowly, if at all. Greiner, Nicholson & Baker (1964) devised a method of treatment depending on the blocking of melanin synthesis by depressing tyrosinase activity. A copper chelating agent, D-penicillamine, was administered for 4 weeks, and four out of six patients improved considerably; this treatment has not been generally accepted.

ADRENOCORTICOTROPHIC HORMONE (ACTH)

ACTH may produce pigmentation of Addisonian pattern. Cass *et al.* (1964), in a series of 122 multiple sclerosis patients treated with ACTH in high doses, found that six developed increased pigmentation. They noted a superimposition of Addisonian abnormalities upon an over-all Cushgoid state. The hypermelanosis was reversed by reduction of the dose of ACTH and by the administration of dexamethasone. Khan & Smith (1970) found an apparently higher incidence of hypermelanosis with the use of the synthetic compound tetracosactrin.

ORAL CONTRACEPTIVES

All oral contraceptives may produce chloasma, particularly in women who have developed such pigmentation in pregnancy. The incidence of chloasma increases with the length of time that the oral contraceptive is taken. Rice-Wray *et al.* (1962) found a high incidence of chloasma in Mexican women receiving the oral contraceptive. Exposure to sunlight and racial pigmentary response certainly influence the proportion of women conspicuously affected. The mechanism of 'pill' chloasma is uncertain, but probably both oestrogen and progesterone are necessary to produce it (Carruthers, 1966). Men taking oestrogens for prostatic carcinoma may develop nipple pigmentation. Excess melanocyte stimulating hormone has been suggested as a cause, but the pigmentation is not Addisonian in type. Only one patient has been described who developed an Addisonian-like syndrome accompanied by buccal pigmentation, while receiving mestranol (Sotaniemi, Kreus and Kaipainen, 1968).

Chloasma induced by oral contraceptives is usually symmetrical but blotchy. It affects mainly the forehead, the malar eminences and the lower parts of the cheeks. There may be a distinct border, particularly about the scalp margin, eyes and lips, or the pigmentation may fade gradually into the surrounding skin (Carruthers, 1966). It differs from chloasma of pregnancy in fading more slowly on cessation of oral contraception than is usual after parturition (Baker, 1969).

CLOFAZIMINE (LAMPRENE)

Clofazimine is a substituted iminophenazine dye used in the treatment of leprosy. It is active in suppressing the acute inflammatory reactions, such as with erythema nodosum associated with lepromatous leprosy. Browne (1965) recorded in detail the changes in skin colour induced by this drug. Light skinned patients may show a diffuse erythema after 2 weeks of treatment, and even in deeply pigmented negroes the erythema may be visible early in the second month. It first appears on the face, palms and soles. The conjunctivae and buccal mucosae are only slightly affected. Lepromatous lesions are not noticeably more affected than adjacent skin. There may be red staining of underclothes, and the urine is deep red. During the second and third months of treatment, patients may develop blackish-brown or purplish-black coloration, particularly of the leprosy lesions. This latter hyperpigmentation appears to be a mixture of erythema with hypermelanosis (Pettit, 1969). The red pigmentation begins to fade soon after the drug is stopped, but the darker pigmentation is more persistent. These changes may be cosmetically unacceptable, particularly in fair-skinned patients, in whom they are most conspicious.

SULPHONES

Fixed eruptions are not uncommon in deeply pigmented Africans; up to 3% of leprosy patients develope such eruptions with sulphones. More or less symmetrical discrete patches are usually seen, but the melanosis may be diffuse or chloasmal (Browne, 1964). Prolonged treatment with a sulphone may produce a slate-grey pigmentation, particularly of the face (Pettit, 1969).

PHENOLPHTHALEIN

It is well known that phenolphthalein may cause a fixed drug eruption with the consequent hyperpigmentation of the affected areas. Almost universal brown pigmentation has been described following the long continued ingestion of this drug (Weiss & Kile, 1935).

OTHER DRUGS

Isolated incidences of alteration in pigmentation have been described with other drugs. Imipramine produced in one patient a purplish discoloration, resembling that provoked by chlorpromazine (Hare, 1970). Methysergide, a drug used in the treatment of migraine, has been reported to product a thickened, reddened 'orange-peel' skin in some patients (Graham, 1964). Nicotinic acid, when administered in large doses, may cause brown pigmentation (Goldsmith, 1965). A blue-black pigmentation appeared on the exposed areas of the face and arms of a man treated with high doses of hydrochlorothiazide for 14 months (Sanatove, 1965). Spillane (1963) reported that brown hair may be bleached to a light golden-brown after 2–3 months of Mephenesin therapy. After discontinuation of the drug, affected hairs regained their previous colour; there was no pigmentation of the skin.

The Middlesex Hospital, ASHLEY LEVANTINE
London, W.1.
The Enfield Group of Hospitals JOHN ALMEYDA

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News and Notices

All enquiries regarding subscriptions to the *British Journal of Dermatology* should be sent to the Publishers, Blackwell Scientific Publications Ltd, Osney Mead, Oxford OX2 0EL.

Institute of Dermatology
(University of London)

Applications are invited from those wishing to be considered for inclusion in the Diploma in Dermatology Course commencing 2 October 1974. Applications should be submitted before 31 December 1973, and applicants will be notified in March 1974 as to whether or not their application has been successful. Application forms may be obtained from the Dean, Institute of Dermatology, St John's Hospital for Diseases of the Skin, Lisle Street, Leicester Square, London WC2H 7BJ.

Psoriasis Association Research Fund

The Committee of the Psoriasis Association is prepared to receive requests for funds for research and apparatus, to be used in connection with research into psoriasis.

Requests should be sent to: The Secretary, The Psoriasis Association, 22 Billing Road, Northampton, NN1 5AT.

International Society of Tropical Dermatology

The International Society of Tropical Dermatology is the only international society devoted to encouraging the study and investigation of skin and venereal disease in the tropics. It is concerned with the geographical ecology of disease on a global basis and with basic research.

The official organ of the Society is the bi-monthly *International Journal of Dermatology.*

The Society has sponsored World Congresses at Naples (1962), Kyoto (1969) and Sao Paolo (1974), and Mycology Symposia at Bratislava (1966) and Bucharest (1973). Other activities are planned.

Enquiries and applications for membership may be addressed to: The Secretary General, Dr Frederick Reiss, 19 East 80th Street, New York, U.S.A.

Dermatological Society of South Africa. Third International South African Dermatological Congress—1975

This congress will be held in Durban, Wednesday–Saturday, 18–21 June 1975.

The main themes of the congress will include: Progress in Dermatological Therapy; Aspects of Tropical Dermatology; Immunology and Cutaneous Malignancy.

Other sessions will be devoted to free (miscellaneous) communications of short duration. International participation is invited and the official language will be English. Further details available from: Dr B. Bentley-Phillips, 708 Eagle Building, Murchies Passage, West Street, Durban, South Africa.

APPOINTMENT
Dr Conrad James Guerrier, M.B., M.R.C.P.

Dr Conrad Guerrier has been appointed Consultant Dermatologist to the Northampton and Kettering groups of hospitals.

He qualified at Guys Hospital Medical School, M.B., B.S., in 1962 and M.R.C.P. (London) in 1968. He received his dermatological training firstly as House Physician to Dr Forman and Dr Moynahan at Guys Hospital, was Registrar to Dr Vickers, and latterly to Dr Ryan, at Oxford and, finally, as MRC

Anglo-French Research Scholar at the Foundation Adolphe de Rothschild in Paris with Professor Michel Prunieras and Dr Ch. Grupper.

Dr Guerrier is an electron microscopist with a particular interest in pigmentary disorders, and has published original material in both French and English in the British, North American and Continental medical press.

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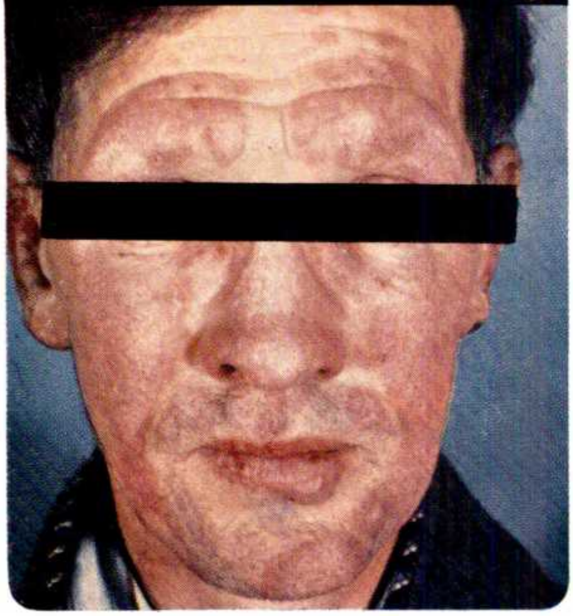

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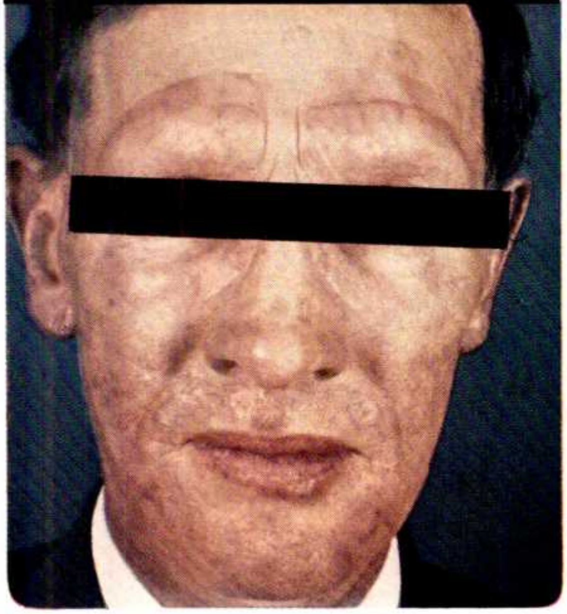

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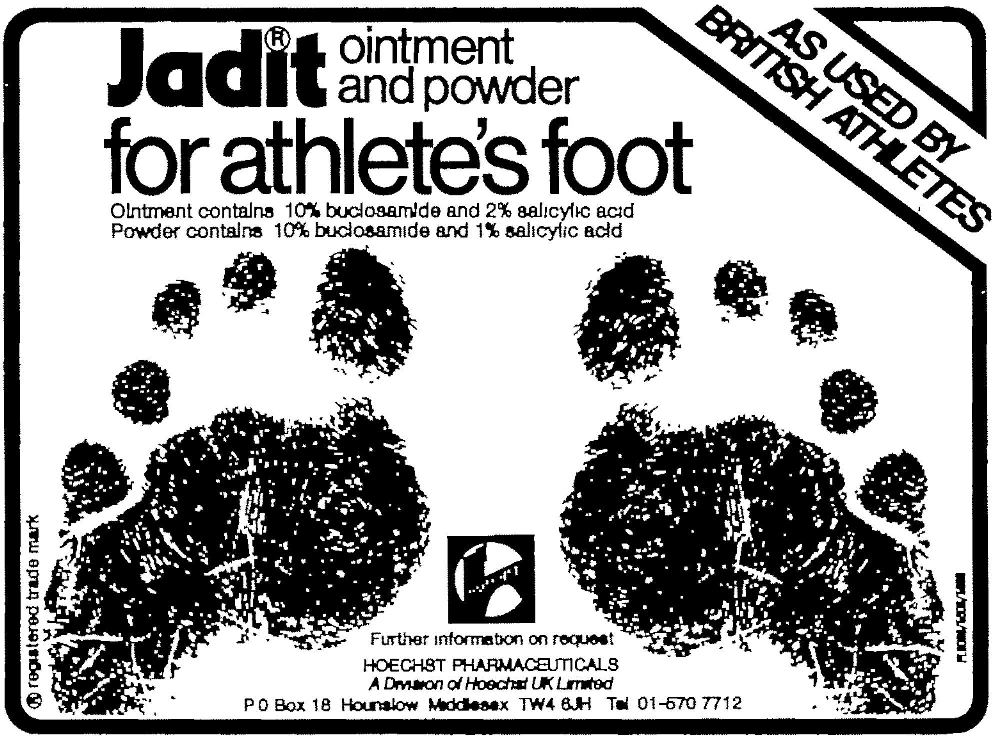

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Clinical Allergy Edited by J. Pepys

Volume 3, Number 2, June 1973

Geoffrey Taylor and Jane Walker. Charles Harrison Blackley, 1820–1900

H. A. Hyde. Atmospheric pollen grains and spores in relation to allergy. II

A. Margaret Hughes and Kate Maunsell. A study of a population of house dust mite in its natural environment

A. Watt, R. Morley, M. Greenberg and A. J. Fox. Follow-up of a group of workers exposed to dusts containing derivatives of *Bacillus subtilis*

J. Pepys, I. D. Wells, M. F. D'Souza and M. Greenberg. Clinical and immunological responses to enzymes of *Bacillus subtilis* in factory workers and consumers

E. S. K. Assem and Monica K. McAllen. Changes in challenge tests following hyposensitization with mite extract

M. F. D'Souza, J. Pepys, I. D. Wells, E. Tai, F. Palmer, B. G. Overell, I. T. McGrath and M. Megson. Hyposensitization with *Dermatophagoides pteronyssinus* in house dust allergy: a controlled study of clinical and immunological effects

Ikuro Kimura, Yoshiaki Moritani and Yoshiro Tanizaki. Basophils on bronchial asthma with reference to reagin-type allergy

Ellen E. E. Jarrett and J. W. Kerr. Threadworms and IgE in allergic asthma

Heather M. Dick, Christine O. Dawson and J. D. Campbell. Farmer's lung: a comparison of simple diagnostic techniques and antigen preparation in human and bovine disease

T. G. Villar, Ramiro Ávila and A. Telles de Araújo. Pulmonary granulomatoses due to inhaled particles: personal experience and some immunological considerations

Valerie Holford-Strevens. Allergenic activity of cat and dog skin fractions obtained by Sephadex gel filtration

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microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	percent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
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British Journal of Dermatology, Volume 89, Number 1, July 1973

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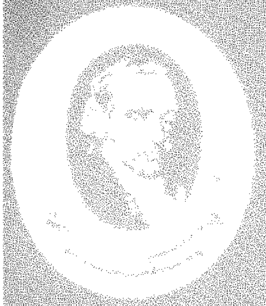

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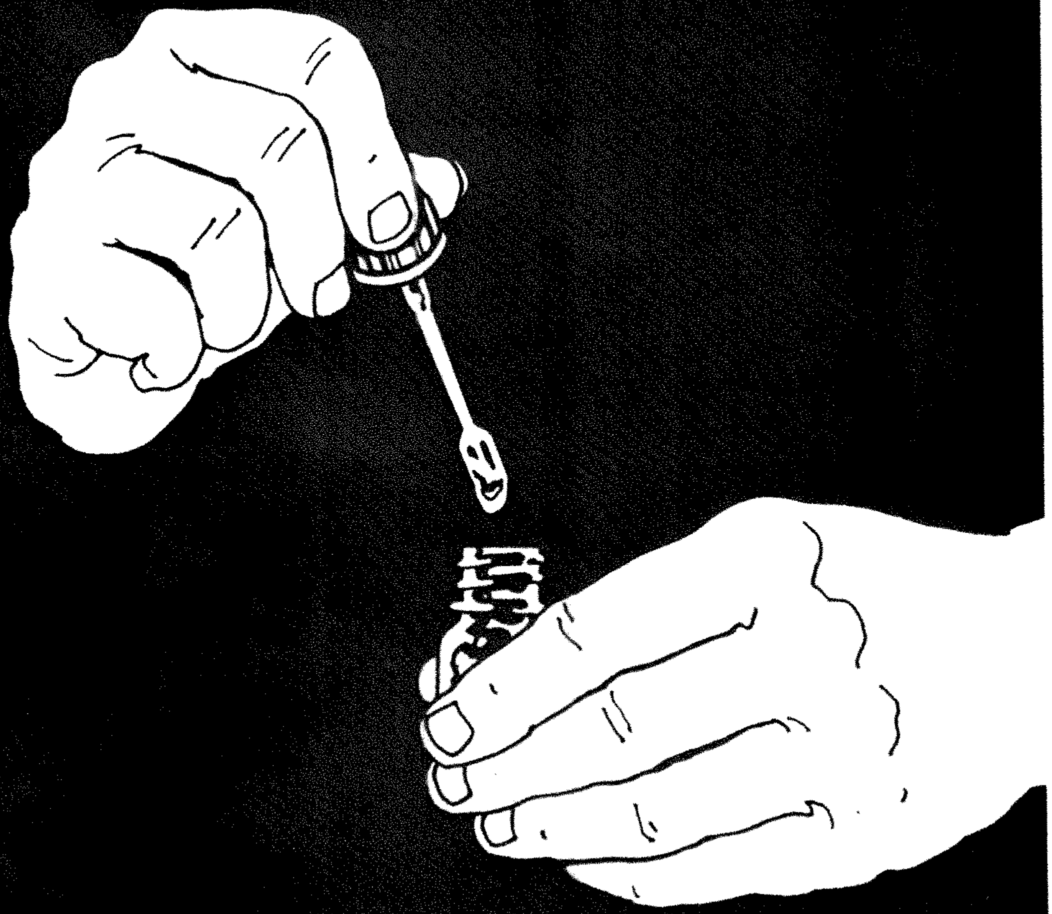

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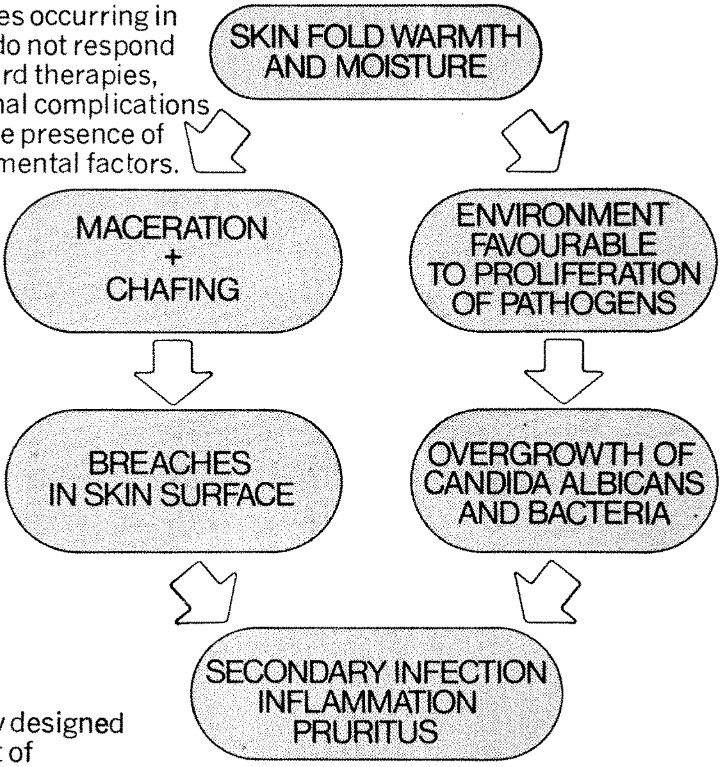

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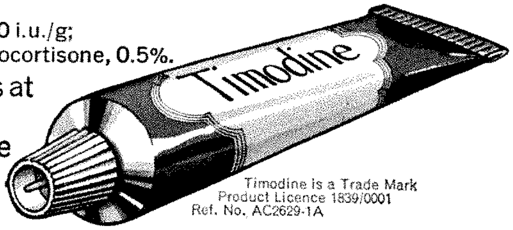

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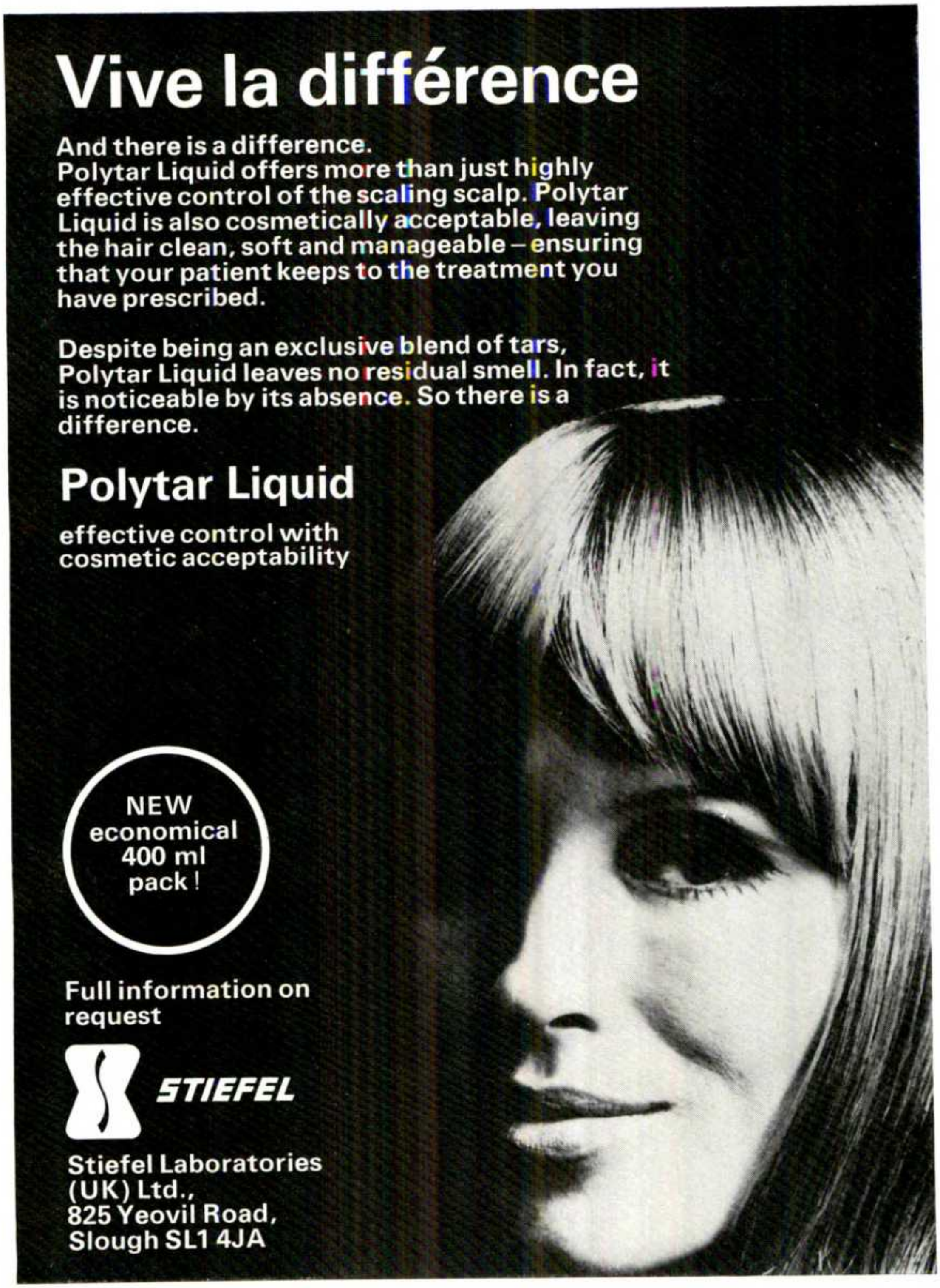

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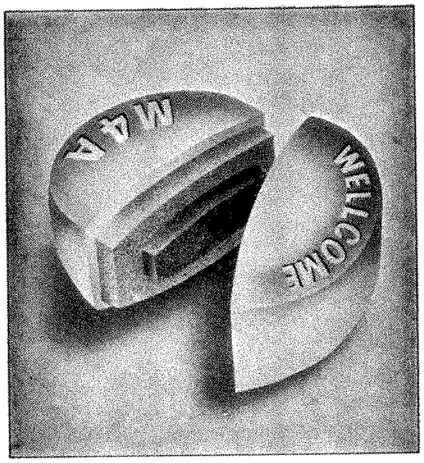

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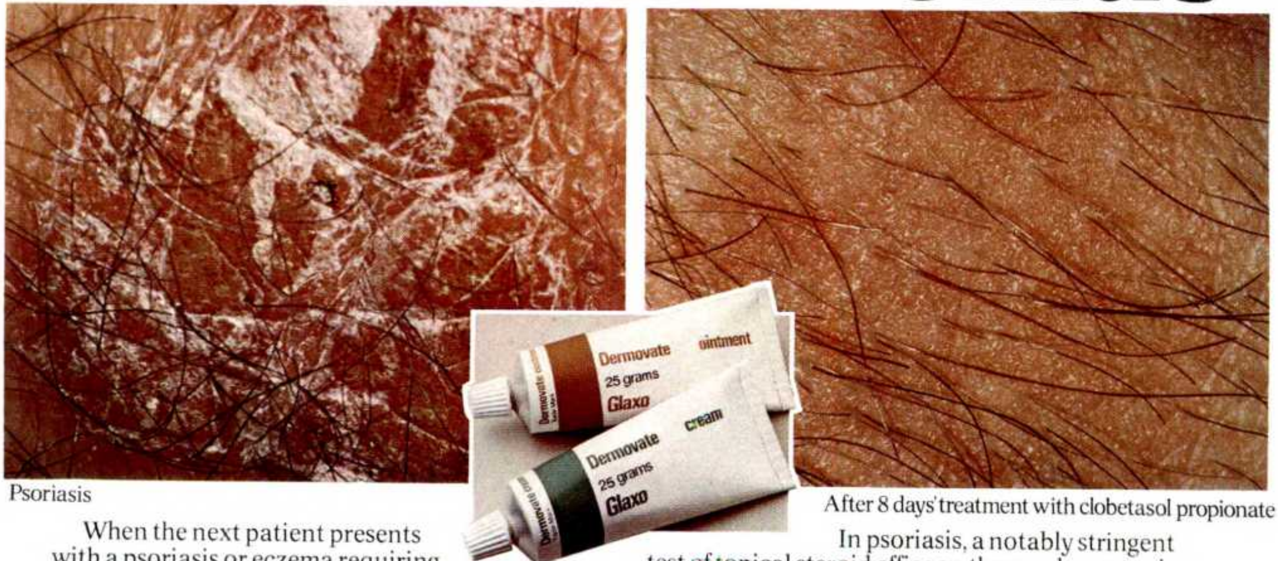

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British Journal of Dermatology (1973) **89**, 115.

Clinical and Laboratory Investigations

Intercellular antibodies in blood and epidermis

A HISTOCHEMICAL STUDY OF IgG IMMUNOGLOBULINS IN PATIENTS WITH LATE REACTIONS TO PENICILLINS AND THEIR COMPARISON WITH SIMILAR ANTIBODIES IN PATIENTS WITH PEMPHIGUS VULGARIS

MICHAEL J.FELLNER,† KIMIE FUKUYAMA,* ALAN MOSHELL
AND MARIA V.KLAUS

Department of Dermatology, New York University School of Medicine, New York,
and *Department of Dermatology, University of California, San Francisco, California, U.S.A.

Accepted for publication 23 February 1973

SUMMARY

Antibodies directed against intercellular epithelial structures (ICE) have been important in the diagnosis of pemphigus vulgaris. Our study of thirty-seven patients with maculopapular eruptions following therapy with penicillins, clearly indicate that antibodies morphologically indistinguishable from those found by standard techniques in pemphigus vulgaris are also found here. Indirect staining by fluorescence and horse-radish peroxidase indicated localization against ICE identical to that seen in pemphigus, in about half the patients. Direct staining of skin from two penicillin-allergic patients revealed similar staining in the familiar pattern, which closely resembles the network of lines in giraffe skin. Detection of circulating antibody directed against a component of epithelium introduces new insight into the pathophysiology of maculopapular penicillin eruptions, although further work is required to show what the exact relationship is to pemphigus vulgaris, the potentially fatal skin disease.

Since the discovery of IgG anti-epithelial antibodies in pemphigus vulgaris (Beutner & Jordon, 1964; Beutner *et al.*, 1965), similar antibodies have been reported in patients with burns (Ablin *et al.*, 1969), drug eruptions (Chorzelski, Jablonska & Blaszczyk, 1966; Fellner & Prutkin, 1970), and in some patients taking sulphamethoxypyridazine (Kynex®) who did not have eruptions (Go, 1969). Very recently, in a case of true pemphigus vulgaris, the question of whether the disease was induced by therapy with D-penicillamine has been raised (Hewitt *et al.*, 1971). Findings such as these suggest the possibility that denaturation of epidermal proteins by heat or drugs might engender the production of antibodies against components of the epithelium. Previous study by electron microscopy and fluorescence techniques showed similarities between skin from patients with maculopapular penicillin eruptions and skin from patients with pemphigus vulgaris (Fellner & Prutkin, 1970). In the present study we used immunohistochemical techniques to investigate circulating antibodies directed to epithelium in a large series of patients with maculopapular reactions following penicillins, in an attempt to understand better the basic immunopathogenic mechanisms involved.

---

† Present address: New York Medical College, Bird S. Coler Hospital, Dermatology Department, Welfare Island, N.Y. 10017, U.S.A.

MATERIALS AND METHODS

Patients

A total of sixty patients was studied. They ranged in age from newborn infants to adults 76 years old. Thirty-seven had clearly defined maculopapular penicillin eruptions, three had pemphigus vulgaris proven by biopsy, and twenty were control subjects. Of the latter, twelve had received penicillin treatment without adverse reactions, while eight, including five newborns, had had no treatment with penicillin that we could detect, either by our history obtained directly from the patients or by review of their records. All patients with penicillin eruptions were seen personally by one (M.J.F.) or more of us. The diagnosis of penicillin eruption was made clinically in all cases, because either the patient was taking only a penicillin, or was taking a penicillin and drugs like digoxin, milk of magnesia, or vitamins, which drugs were unlikely to have produced the maculopapular eruptions. One patient known to have infectious mononucleosis developed an extensive and intense maculopapular eruption only after treatment with ampicillin for septicaemia. In this patient we concluded that the ampicillin rather than the mononucleosis accounted for the rash, because of the interval after drug therapy before the eruption began and of the intensity of the eruption. The patients were being treated with penicillins for infectious diseases—respiratory infections, osteomyelitis, urinary tract infections, subacute bacterial endocarditis, and syphilis. Three patients were continued on therapy despite their eruptions, albeit with lower drug dosages, and in combination with antihistamines and topical corticosteroids. Their skin lesions cleared in several days, despite continuation of the drugs. Another patient with a maculopapular eruption following ampicillin was followed over a year and is described below.

The diagnosis in the three patients with pemphigus vulgaris was proven by histological examination of skin lesions.

Immunofluorescence technique

Guinea-pig oesophagus was routinely used as the substrate for indirect immunofluorescence staining. Male albino guinea-pigs were sacrificed and the oesophagus was freed, wrapped in tin foil, quick-frozen in liquid nitrogen and cut in a cryostat into sections 4 μm thick. Sections were dried in air and used immediately or stored at −20°C for no longer than a week. Sera from patients with penicillin eruptions or pemphigus and from control subjects were separated from clotted blood and stored at −20°C until used.

Guinea-pig sections were washed once in phosphate-buffered saline (PBS) pH 7·5, overlaid with diluted serum for 30 min, rinsed with a few millilitres of PBS, and placed in a Coplin jar and washed with PBS for 10 min. Goat anti-human IgG labelled with fluorescein isothiocyanate was then applied for 30 min and the sections were incubated in a moistened chamber. The slides were rinsed and washed again for 60–90 minutes in PBS, and the sections were mounted in buffered glycerol. Fluorescein-labelled anti-IgG with a fluorescein/protein (F/P) ratio of approximately 2·8 and an antibody content of 50 μg/ml was kindly provided by Dr Ernst Beutner. We also used commercially obtained (Hyland Laboratories) fluorescein-labelled anti-IgM and anti-IgG with an F/P ratio of 2·8.

Direct immunofluorescence staining was performed on punch biopsies obtained from the skin lesions. The specimens were quick frozen, cut at 6 μm and stained with the above-mentioned technique, but omitting prior incubation in serum.

Readings of sections in duplicate were done independently by two, and sometimes three, individuals. Staining was graded as follows: negative, no stain: 1+, faint green stain between cells; 2+, moderate green stain between cells; 3+, strong green stain between cells.

Peroxidase technique

Frozen guinea-pig lip sections were obtained and treated with sera of patients as described above. They were overlaid with peroxidase-labelled goat anti-human IgG prepared by the method of Nakane & Pierce (1965, 1969) instead of the fluorescein conjugate. The tissues were then washed with PBS and were treated with a saturated solution of 3,3-diaminobenzidine containing an 0·05 M tris buffer, 0·0025% hydrogen peroxide of pH 7·6, for 15 min to stain sites to which the peroxidase-labelled antibody was bound. Osmium tetroxide solution (2%) was then placed on the section for 1 h to enhance the reaction product. The sections were dehydrated in alcohol, cleared in xylene, and mounted in Permount.

Microscope

A Leitz Ortholux microscope was used for fluorescence microscopy; equipped with an Osram mercury ultraviolet lamp, 2 mm UG-1 exciter filter, 2·6 mm Euphos barrier filter, and a darkfield condenser.

Skin tests, haemagglutination tests, and blood typing

The methods for detecting antibodies of various specificities of penicillin derivatives were previously described (Levine & Ovary, 1961; Parker *et al.*, 1962; Levine, Fellner & Levytska, 1966). In brief, aqueous, potassium penicillin G, 0·02 M, 10,000 units/ml was used in testing for 'minor' determinants. Penicilloyl-polylysine 6×10^{-5} M was used for detection of the 'major' determinants. This was kindly supplied by the Kremers-Urban Company, Milwaukee, Wisconsin. Skin reactions for immediate weal-and-flare were read in 15–20 min. A distinct weal larger than 6 mm with a clear flare 10 mm or more in diameter was considered positive. Delayed skin test reactivity was tested for with aqueous potassium penicillin G, 0·1 M, approximately 50,000 units/ml, and was read in 24–48 h as erythema and induration.

Haptenic specificity of the antibodies of the immediate type has been fully described (Parker *et al.*, 1962; Levine, Fellner & Levytska, 1966; Levine *et al.*, 1966) both for the weal and flare reaction and the haemagglutinating antibodies. Blood typing was done by minor cross-match using patient's serum.

RESULTS

Indirect immunofluorescent staining

Sera taken from thirteen out of twenty patients with morbilliform eruptions following administration of penicillins showed staining at titres of 1/10 and 1/20 localized in the intercellular area of epithelium of guinea-pig oesophagus. Sera from seven of the twenty patients showed no staining. Table 1 summarizes the results. Of the thirteen positives, seven had received only ampicillin, two each only penicillin G or procaine penicillin, one phenoxymethyl penicillin, and one both ampicillin and oxacillin. Sera from eight control patients showed no fluorescent staining. Five of these eight controls had received penicillin recently without adverse reaction.

Sera from three of the patients with morbilliform eruptions showing staining localized to the intercellular area were serially diluted until the staining was no longer present. These end point titrations gave results of 1/60, 1/100 and 1/160 respectively.

Sera from all three patients with pemphigus vulgaris were positive at titres of 1/20, 1/40 and 1/80.

TABLE 1. Indirect immunofluorescent studies in twenty patients with maculopapular penicillin eruptions

Subject	Age	Sex	Type of penicillin*	Skin tests†		Antiepithelial‡ antibody
				Immediate	Delayed	
1	22	M	Ampicillin	–	Not done	3+
2	48	F	Ampicillin and oxacillin	–	+	2+
3	19	F	Ampicillin	–	–	2+
4	49	M	Ampicillin	–	Not done	2+
5	48	M	Procaine	Not done	Not done	2+
6	33	M	Phenoxymethyl	–	Not done	1+
7	75	M	Penicillin G	–	Not done	1+
8	19	M	Ampicillin	–	–	1+
9	69	F	Ampicillin	–	–	1+
10	49	M	Penicillin G	–	–	1+
11	67	M	Ampicillin	–	–	1+
12	23	M	Ampicillin	–	+	1+
13	51	F	Procaine	–	Not done	1+
14	76	M	Ampicillin and oxacillin	–	–	–
15	40	F	Ampicillin	–	–	–
16	49	M	Ampicillin	–	–	–
17	40	F	Ampicillin	Not done	Not done	–
18	61	M	Ampicillin	–	Not done	–
19	58	M	Penicillin G	–	–	–
20	7	F	Penicillin G	–	–	–

Eight controls had no staining; five out of eight had received penicillin without adverse reactions, three out of eight had received no penicillin.

* See text for description of dosages and routes of administration.

† Skin tests for immediate and delayed reaction to penicillin derivatives.

‡ All serum dilutions were done initially at 1/10 and 1/20, and are reported here at 1/20.

Direct fluorescence staining

Direct fluorescence staining was carried out on skin samples from three patients with morbilliform penicillin eruptions. Two showed epithelial intercellular staining specific for IgG. Direct staining for IgM was negative in all three patients.

Horse-radish peroxidase staining

Sera were studied from thirty-five patients with maculopapular eruptions after penicillins. The results are given in Table 2. At 1/10 dilutions, fifteen of the thirty-five sera showed staining localized in intercellular positions (Fig. 1) identical to that seen by immunofluorescence technique. The staining patterns were also comparable to those observed with sera of patients with pemphigus vulgaris (Fig. 3). Twenty of the thirty-five sera elicited no staining. The intensity of staining was graded as before. Eighteen were the sera studied by immunofluorescence techniques.

There was a lack of staining using serum from a patient who had no eruption following treatment with penicillin. Serum of one of the twenty control subjects elicited a 1+ stain by the peroxidase method. This patient, a 58-year-old man with psoriasis of long duration, had received penicillin as

TABLE 2. Indirect staining by horse-radish peroxidase in thirty-five patients with maculopapular penicillin eruptions

				Skin tests*		
Subject	Age	Sex	Type of penicillin	Immediate	Delayed	Antiepithelial† antibodies
1	54	F	Ampicillin	–	–	2+
2	19	M	Ampicillin	–	–	1+
3	69	F	Ampicillin	–	–	1+
4	48	M	Procaine	–	+	1+
5	8	M	Penicillin V	–	–	1+
6	49	M	Penicillin G	–	–	1+
7	19	F	Ampicillin	–	Not done	1+
8	75	M	Penicillin G	–	Not done	2+
9	33	M	Phenoxymethyl	–	Not done	1+
10	67	M	Ampicillin	–	–	1+
11	51	F	Unknown	–	Not done	2+
12	28	M	Procaine	–	Not done	1+
13	60	F	Procaine	+	Not done	1+
14	29	M	Penicillin G	–	–	2+
15	53	M	Phenoxymethyl	+	Not done	1+
16	40	F	Ampicillin	Not done	Not done	–
17	8	F	Ampicillin	Not done	Not done	–
18	27	M	Benzathine	–	–	–
19	51	F	Procaine	–	Not done	–
20	76	M	Ampicillin and oxacillin	–	–	–
21	49	M	Ampicillin	–	Not done	–
22	58	M	Penicillin G	–	–	–
23	40	F	Ampicillin	–	–	–
24	7	F	Penicillin	–	–	–
25	20	F	Procaine	–	–	–
26	74	M	Penicillin G	–	–	–
27	76	M	Penicillin G	–	–	–
28	66	F	Ampicillin	+	Not done	–
29	70	M	Procaine	–	–	–
30	48	F	Ampicillin	–	+	–
31	49	M	Ampicillin	–	–	–
32	61	M	Ampicillin	–	Not done	–
33	57	F	Penicillin	–	Not done	–
34	60	M	Unknown	+	Not done	–
35	10	F	Penicillin	–	Not done	–

* Skin tests for immediate and delayed reaction to penicillin derivatives.

† Serum dilutions were 1/10.

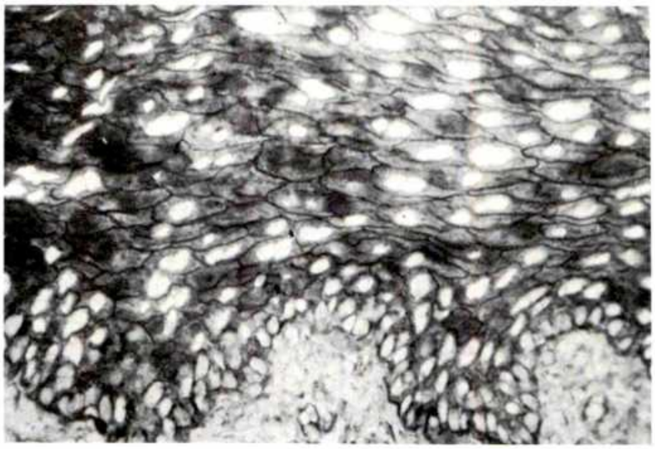

FIGURE 1. Indirect staining with horse-radish peroxidase on serum from a patient with a morbilliform penicillin eruption.

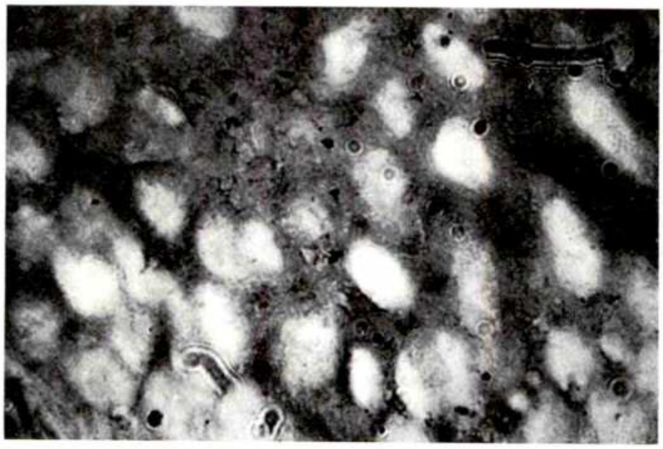

FIGURE 2. Staining with horse-radish peroxidase, using serum from a control patient with no eruption following penicillin. The stain and substrate material are the same as in Figs. 1 and 3.

therapy 13 years previously, but did not recall any recent treatment with penicillin. It is noteworthy that the patient had a positive delayed skin test to penicillin G, the only positive delayed skin test among eight of the twenty controls so tested. Table 3 summarizes findings with staining by horse-radish peroxidase-conjugated and fluorescence-conjugated IgG in eighteen patients with maculopapular eruptions from penicillins. Results were almost identical. Table 4 compares the results obtained from patients with maculopapular eruptions to those from patients with no eruptions. The differences were statistically significant ($0{\cdot}01 > P > 0{\cdot}001$).

Blood types

Of the thirteen patients whose sera were positive by indirect fluorescence, two had type B blood, three type A, and eight type O. Among the fifteen patients with negative sera, eleven had type O blood, three type B, and one type AB.

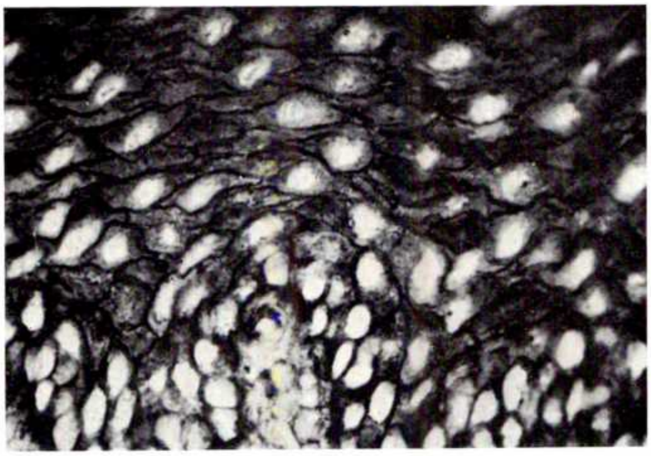

FIGURE 3. Indirect horse-radish peroxidase staining with serum from a patient with pemphigus vulgaris using guinea-pig lip as substrate. Intercellular localization of IgG is seen.

TABLE 3. A comparison of staining by horse-radish peroxidase and fluorescence in eighteen patients with maculopapular eruptions from penicillins*

			Grading of staining	
Patient	Age	Sex	Horse-radish peroxidase	Fluorescence
1	19	M	1+	1+
2	69	F	1+	1+
3	48	M	1+	2+
4	49	M	1+	2+
5	19	F	1+	2+
6	75	M	2+	1+
7	33	M	1+	1+
8	67	M	1+	1+
9	51	F	2+	1+
10	40	F	–	–
11	51	F	–	–
12	76	M	–	–
13	49	M	–	–
14	58	M	–	–
15	7	F	–	–
16	40	F	–	–
17	49	M	–	–
18	61	M	–	–

* Due to limitations imposed by the quantities of serum involved, only eighteen sera could be examined by both techniques. Horse-radish peroxidase staining alone was done on seventeen additional patients, and fluorescence staining alone was done on two additional patients.

TABLE 4. Intercellular antibodies seen by fluorescence and/or peroxidase in thirty-seven patients who had received penicillins and developed maculopapular eruptions, and in twenty patients without eruptions*†

Patients	Indirect stain positive	Indirect stain negative
With maculopapular eruptions	48% (17/37)	52% (20/37)
With no eruptions	5% (1/20)	95% (19/20)

* Of those twenty patients without eruptions, six had received penicillin within a year, six had received penicillin 1–15 years previously, and eight knew of no therapeutic exposure to penicillin.

† $0.01 > P > 0.001$, analysed by fourfold contingency.

Skin tests

Four of the patients with penicillin-induced morbilliform eruptions showed immediate sensitivity to penicillin derivatives. Of these, two patients showed intercellular staining, while the other two did not reveal the presence of this staining (Tables 1 and 2).

Similarly, three of the patients with penicillin-induced morbilliform eruptions showed delayed hypersensitivity to penicillin derivatives. Of these, two patients showed intercellular staining, while the other did not (Tables 1 and 2).

Haemagglutination

The haemagglutination titres of all the patients in the study were uniformly low or absent, and there was no difference in the average titres between any single group of patients as compared to any other group, or to the average titre of all the patients studied.

Report of a case

One patient, a medical student, was studied in detail and followed-up for 14 months after suffering a maculopapular ampicillin eruption. The student, an intelligent 23-year-old male, took 1 g ampicillin over the course of 2 days, in the form of tablets, as therapy for a respiratory infection. No other drugs

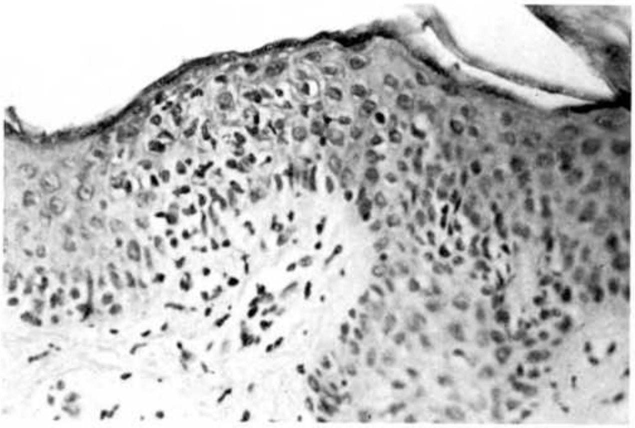

FIGURE 4. A section of skin from a patient with a florid maculopapular eruption following ampicillin, stained with haematoxylin and eosin. Mild intercellular oedema is seen in the epidermis.

TABLE 5. Fluorescence staining in a patient with a morbilliform eruption after α-amino benzylpenicillin

	Indirect	Direct
During eruption	Positive (1/100)	Positive
1 month after eruption	Negative	Not done

were taken. 6 days later he developed a generalized morbilliform eruption. A skin biopsy was taken during the acute stage. Sections of the epidermis stained with haematoxylin and eosin showed areas of mild intercellular oedema and exocytosis. The upper dermis showed a mild perivascular infiltrate of lymphocytes and histiocytes (Fig. 4). Staining of his skin by direct fluorescence technique was positive, revealing IgG localized between epithelial cells. These results, as well as those of indirect staining, using his serum on guinea-pig skin, are summarized in Table 5. Skin tests for immediate weal-and-flare reactions to 'major' and 'minor' derivatives of penicillin were performed as described previously, and were both negative. Skin testing for delayed hypersensitivity with 0·1 M penicillin G revealed a 1+ positive test at 24 and 48 h, with erythema and induration of 6 mm in diameter. The titre of penicilloyl-specific antibodies detected by haemagglutination was never greater than 1/8, and was of the IgM class. The patient's eruption cleared within a week and he has remained free of cutaneous lesions for the last 14 months of follow-up. During this time he has been seen by us at 2-week intervals to check for recrudescence or development of any skin lesions. The patient had taken penicillin 4 years previously without any adverse reaction. He had no personal history of atopy or drug sensitivity and was in good health except for obesity. His father had had an anaphylactic reaction after an injection of penicillin many years ago. A 20-year-old sister had had a similar morbilliform reaction following ampicillin. His other siblings, aged 6, 18, and 21, had had no adverse reactions to penicillin.

DISCUSSION

The results of these experiments indicate that about a half of patients suffering from morbilliform drug eruptions following therapy with penicillins, and especially ampicillin, exhibit in blood IgG antibodies directed to the intercellular area of the epithelium, which appear to be similar to those seen in patients with pemphigus vulgaris. After years of research, the immunopathogenic mechanism producing the maculopapular penicillin eruptions still remains unknown. Rising titres of penicilloyl-specific IgM antibodies were detected in the sera of a small number of patients, suggesting the possibility that the eruptions were initiated by antigen-IgM-antibody complexes localized in vessel walls as in experimental serum sickness (Levine *et al.*, 1966). Yet serum sickness-like reactions to penicillin in man are usually accompanied by urticaria rather than maculopapular eruptions. Immunologically, such patients have positive immediate skin tests involving IgE antibodies of various penicillin specificities, and sometimes IgG as well, but they have either no penicilloyl-specific IgM or else very low titres of these antibodies. Delayed hypersensitivity has also been thought to play a role in the initiation of some of these maculopapular eruptions, which are the most common form of adverse reaction seen after the penicillins (Fellner *et al.*, 1969).

In view of the lack of any significant circulating antibody directed against the penicillin molecule as determined by the haemagglutination method, and the lack of any difference between groups in the low titres of haemagglutinating antibodies detected, it seems unlikely that the antibody detected by the immunofluorescence and horse-radish peroxidase techniques could specifically bind penicillin. Furthermore, the existence of heteroligating antibodies, in this case to penicillin and intercellular substance, is thought to be extremely unlikely.

Detection of circulating antibody directed to a component of epithelium introduces new insight into the immunopathogenesis of the maculopapular penicillin eruptions. The induction of antibodies by drugs in man is a well known phenomenon, perhaps best exemplified by the report of eight out of sixteen patients taking procainamide, who developed antinuclear antibodies in their blood (Blomgren *et al.*, 1969). Penicillin G forms numerous links with epidermal proteins, resulting in several distinct haptenic groupings (Levine & Ovary, 1961; Parker *et al.*, 1962). Ampicillin, with a tendency to form aggregates more readily than penicillin G, may have a particular affinity for the epidermal structure. Denatured epidermal proteins thus produced might become antigenic, and stimulate the antibody characteristically described in patients with pemphigus vulgaris.

Circulating antibody which reacts with the intercellular area of the epithelium has also been reported in patients suffering from extensive burns (Ablin *et al.*, 1969; Thivolet, Beyvin & André, 1970; Chorzelski *et al.*, 1971). In these patients it would be interesting to determine how many have also been treated with antibiotics or other drugs, since the skin may have been denatured not only by the heat, but also by the drugs. A combination of the two factors may also facilitate production of anti-epithelial antibodies. The denatured proteins produced by the burn could be rendered more antigenic by coupling with chemical drugs.

Thivolet *et al.* (1970) also described antiepithelial antibodies in patients with Lyell's toxic epidermal necrolysis, although this has not yet been confirmed by others.

IgG antibody localized in the skin of patients afflicted with maculopapular eruptions from penicillin was also shown by direct immunofluorescence microscopy, indicating that *in vivo* antigen–antibody reactions occurred in these patients. Ultrastructural changes characteristic of the skin lesions might have resulted from an immunoreaction identical to that occurring in the induction of lesions in pemphigus vulgaris, although acantholysis has been reported to be produced by a simple chemical, as seen in contact dermatitis due to gold (Malten & Mali, 1966; Fox, Kennedy & Rostenberg, 1961). It may be possible, therefore, that morbilliform penicillin eruptions and pemphigus vulgaris have the same pathogenetic mechanism. In patients with penicillin eruptions, however, the appearance of antibodies detected to date (in three patients followed-up for several months) has been uniformly transient, while in cases of untreated pemphigus vulgaris the antibodies tend to persist longer. End-point titrations carried out on the sera of three patients with penicillin eruptions gave results of 1/60, 1/100, and 1/160, which were not as high as those reported for pemphigus vulgaris. The comparable results obtained by two established techniques for the detection of intercellular antibody, in eighteen patients examined in a blind manner, further established the morphological identity of the antibodies and the consistency of the findings. Therefore, may it be said that penicillin reactions produce an abortive form of pemphigus vulgaris? In support of this view is the report of the triggering of a case of childhood pemphigoid by ingestion of a sulphonamide (Bean, Good & Windhorst, 1970). As for penicillins, there is a recent report of a case of bullous pemphigoid in a 2½-year-old boy, which appeared to be triggered by treatment with phenoxymethyl penicillin (Fincher, Dupree & Bean, 1971). The question of 'true' pemphigus vulgaris, characterized by acantholytic cells, precipitated by therapy with D-penicillamine was raised by a recent case report (Hewitt *et al.*, 1971).

Another possibility, although unlikely, is that the intercellular reaction is due to blood group antibodies. The immunofluorescent localization of blood groups A and B antigens was found at the intercellular area (Szulman, 1960, 1962) In our study the distribution of blood groups among patients with positive reactions did not differ from that among patients with negative reactions.

Antibodies to the intercellular area of the epithelium were produced experimentally in animals by Grob & Inderbitzen (1967) with an insoluble fraction of rabbit oesophageal mucosa, and by Ablin & Beutner (1969) with an ethanol-soluble fraction of bovine oesophagus. The latter investigators considered the circulating antibody to be 'histologically and immunologically indistinguishable from the intercellular antibodies of pemphigus'. Pass, Janis & Marcus (1971) used human wart tissue to immunize rabbits. The antibodies formed reacted with the surface of epidermal cells, and their illustration by immunofluorescence demonstrated a reaction resembling pemphigus antibody in the intercellular area of the wart tissue.

Our findings indicate clearly that production of antibodies directed to the intercellular area of the ephithelium is not a phenomenon specific for patients with pemphigus vulgaris. We are, therefore, in favour of abandoning the terms 'pemphigus antibody' and 'pemphigus-like antibody'; intercellular epithelial antibodies (ICE) is a preferable designation.

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Suction blisters in bullous pemphigoid and other dermatoses

STANLEY COMAISH AND EVA McVITTIE*

University Department of Dermatology, Royal Victoria Infirmary,
Newcastle-Upon-Tyne 1

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SUMMARY

Separation of the epidermis from dermis to produce blisters by means of suction has been studied in relation to bullous pemphigoid and certain other skin disorders. There is a correlation between the time taken to produce blisters and the activity of pemphigoid and it is suggested that the technique can be of value in following the course of this disease and as a quantitative method for studying epidermodermal adherence *in vivo*.

The physical separation of epidermis from dermis by suction is a simple technique without hazard to the patient and producing little or no discomfort. Several workers have studied it in relation to blistering diseases and Copeman (1970) used suction blisters to monitor the effect of treatment in porphyria.

We have evaluated this method of assessing epidermodermal adherence in patients with bullous pemphigoid, pemphigus, dermatitis herpetiformis, and epidermolysis bullosa simplex, as well as in patients with eczema and psoriasis.

PATIENTS AND METHODS

Patients with pemphigoid were examined at different stages throughout the disease process, and during remission. Patients with other dermatoses were in general tested only during an active phase. Controls were healthy volunteers from medical and research staff and patients with minor non-blistering lesions such as warts.

In all subjects blisters were produced in the clinically uninvolved skin on the volar surface of the forearm at 150 mmHg below atmospheric pressure. The apparatus used consisted of a small perspex chamber which was placed on the skin and kept in position with a lightly applied rubber bandage. The pump used could produce pressures down to 700 mmHg below atmospheric (60 mmHg) and pressure tubing connected the pump to a manifold which permitted the use of up to four chambers at once, either on one or several subjects. A reservoir bottle prevented any sudden alteration in pressure which small occasional leaks might have otherwise produced. The suction chamber contained a perforated disc with seven apertures, each of 5 mm diameter. The skin could be observed through the transparent roof of the chamber and the times of onset of blister formation in each of the seven apertures were noted. The average of these was recorded as the mean blistering time in minutes (MBT).

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* At present Lecturer in Dermatology, University of Edinburgh.

RESULTS

The appearance of the arm at the conclusion of a test is shown in Fig. 1. Histologically, a small cleft appeared between the epidermis and dermis above the seemingly intact basement membrane, well seen in sections stained with Periodic Schiff Reagent (PAS) (Fig. 2.). If suction was maintained this cleft enlarged to form a complete sub-epidermal blister visible to the naked eye. The greater the negative pressure applied the quicker the blisters formed and the speed of onset was also effected by age (Fig. 3). Peachey (1971) has recently emphasized the influence of local skin temperatures on suction blister formation and our own findings confirm his observations (Fig. 4).

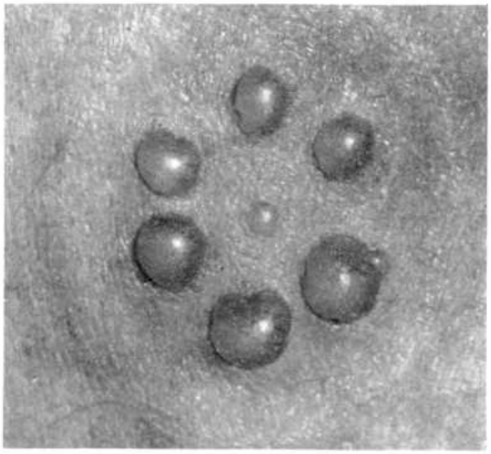

FIGURE 1. Appearance of arm at conclusion of test.

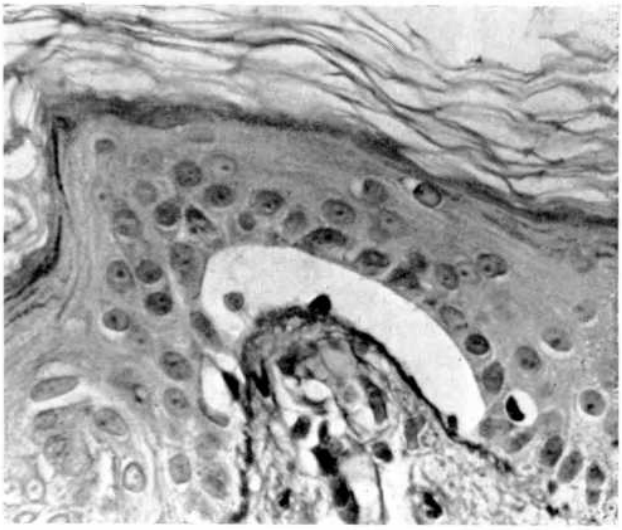

FIGURE 2. Appearance of cleft between epidermis and dermis.

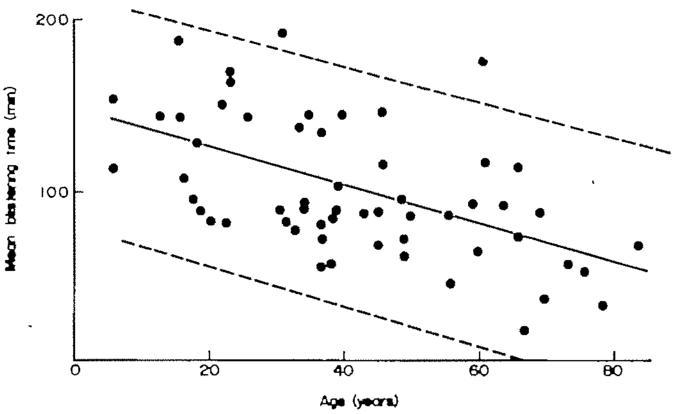

FIGURE 3. The effect of age upon the MBT.

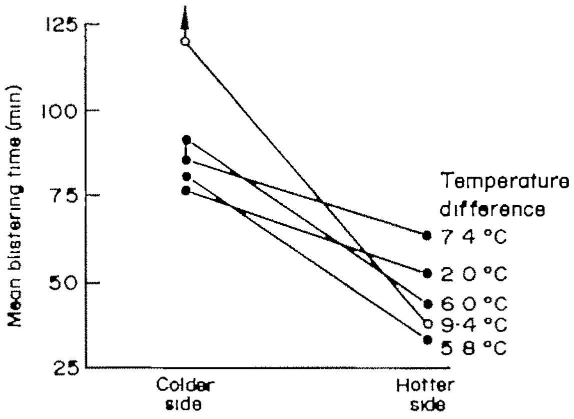

FIGURE 4. The effect of temperature upon the MBT. Suction pressure—150 mmHg. ○, female; ●, male.

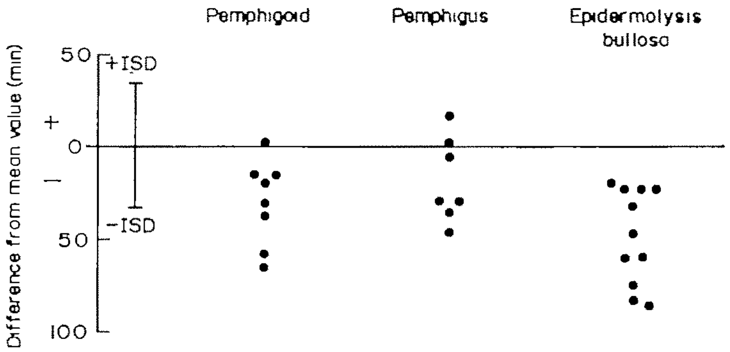

FIGURE 5. Difference in the MBT between pemphigoid, pemphigus and epidermolysis bullosa. Mean values age corrected.

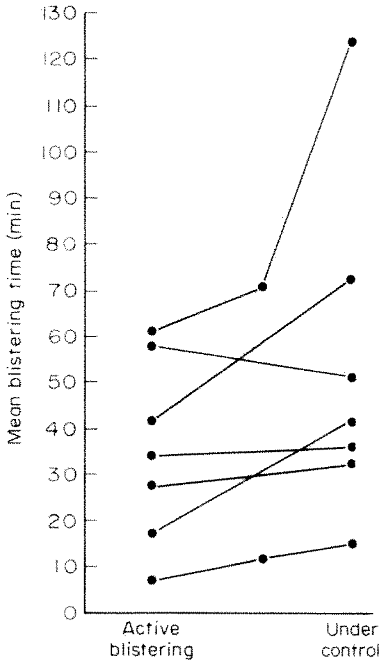

FIGURE 6. Difference in the MBT between active and controlled pemphigoid.

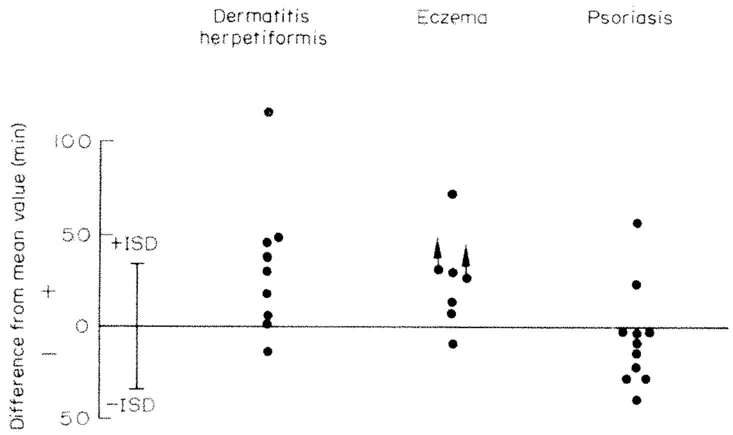

FIGURE 7. Difference in the MBT between dermatitis herpetiformis, eczema and psoriasis. Mean values age corrected.

In bullous pemphigoid the mean blistering time was decreased ($P<0{\cdot}002$), even allowing for the age of the patients, but this was not so with pemphigus ($P>0{\cdot}05$) (Fig. 5). In seven of the patients with pemphigoid the MBT was determined at different stages of clinical activity. In six of these, as the disease came under control the MBT increased (Fig. 6).

In active, but not burned-out, epidermolysis bullosa simplex there was also a significant decrease in MBT ($P<0{\cdot}001$). The values for psoriasis were normal, but those for both eczema ($P<0{\cdot}02$) and dermatitis herpetiformis ($P<0{\cdot}05$) were significantly raised (Fig. 7).

DISCUSSION

The contrast between the results for pemphigoid and pemphigus is of great interest and correlates with the different sites of epidermal cleavage in the two diseases and with the sites of auto-antibody deposition as shown by immunofluorescence. The normal findings with pemphigus suggest that in contrast to pemphigoid, epidermodermal integrity is not grossly affected. In this respect it is noteworthy that histological examination of suction blisters in pemphigus reveals a split in the usual subepidermal site and there is no acantholysis of prickle cells nor any tendency to intraepidermal cleavage as with the spontaneous lesions. A group of patients receiving large doses of corticosteroids over a 5-day period for other (non-blistering) conditions showed no regular alteration of MBT, suggesting that the improvement in MBT induced by these drugs in pemphigoid was not necessarily one example of a general effect on epidermodermal adherence.

The findings with epidermolysis bullosa simplex are wholly in accord with the clinical and histological features of this disorder but the raised MBT values for dermatitis herpetiformis and eczema are surprising and at present we can offer no convincing explanation for these findings.

This simple technique provides an opportunity to study the forces maintaining the integrity of the epidermodermal interface. The derangement of these forces by disease produces a number of distinct and dramatic clinical pictures of which bullous pemphigoid is the outstanding example. The critical role of histological and immunofluorescent techniques in the diagnosis of these diseases is now fully accepted, but we suggest that suction blister studies could assist in assessing the activity of the disease process during the course of therapy. The portable apparatus developed by Kiistala (1969), though somewhat expensive, is admirably suited for such day-to-day measurements in the ward or outpatients.

There are at present two main hindrances to successful clinical exploitation of this technique; one is the time required to raise the blisters and the other is the wide variation in MBT found with normal subjects even when site and age are taken into account. The MBT can be shortened by increasing the applied suction pressure and a log-log relationship between these has been demonstrated (Lowe & van der Leun, 1968). Too rapid blister formation, however, produces haemorrhage which obscures the apertures; shortened blistering times will also tend to reduce the discriminatory value of the test in different conditions. However, a pressure of 300–350 mmHg below atmospheric reduces the MBT to a reasonable convenient period (50–60 min for a 40-year-old adult) and with only occasional haemorrhage.

The wide variation in MBT in normal subjects is not accounted for by any one factor: local skin temperature is undeniably important but even when this is carefully controlled there is on average a 9·1% discrepancy between symmetrical sites on the two arms of a subject at minus 300 mmHg (Peachey, 1971) and variability between subjects is naturally greater. It is evident that at present statistical evaluation of differences between groups of patients gives valuable information, but measurements on individuals must be interpreted cautiously with due regard to the normal variations encountered. Repeated measurements and comparison between symmetrical sites on one subject can clearly be regarded with less reserve.

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The incidence of HL-A antigens in dermatitis herpetiformis

A.G.WHITE, R.ST C. BARNETSON, J.A.G.DA COSTA AND
D.B.L. McCLELLAND

Scottish South East Regional Blood Transfusion Centre and Departments of Dermatology and Therapeutics,
University of Edinburgh

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SUMMARY

In dermatitis herpetiformis (DH) there are small intestinal abnormalities similar to those in adult coeliac disease (ACD) and there is evidence of other similarities between the two conditions, including immunological disturbances.

Two reports of patients with ACD have shown a distinctive distribution of HL-A phenotypes, with a high incidence of HL-A1 and 8. A recent paper has described an increased incidence of HL-A8 in DH.

We report a similar study of patients with DH. Twenty-one of thirty-five patients had HL-A8, an incidence of 60%, as compared to 33% of 175 controls. This difference is significant. The relationship of those patients with HL-A8 to those with abnormal jejunal biopsies, and also to those with circulating auto-antibodies, is discussed.

The pathogenesis of dermatitis herpetiformis (DH) is still unresolved, but the finding of circulating auto-antibodies (Seah *et al.*, 1971), together with quantitative alterations in the serum immunoglobulins (Fraser, Dick & Crichton, 1969) and IgA deposits in the skin (Seah *et al.*, 1972), suggest a breakdown of immunological tolerance.

The relationship of dermatitis herpetiformis to adult coeliac disease (ACD) is not clear. A high proportion of patients with DH have abnormalities of the jejunal mucosa similar to those found in ACD, and in some cases of DH the small intestine reverts to normal after the withdrawal of gluten from the diet (Fry *et al.*, 1968; Marks & Whittle, 1969). There may also be an immunological abnormality common to the two conditions, as in both, the small intestinal immunoglobulin levels are elevated (Asquith, Thompson & Cooke, 1970; McClelland *et al.*, 1972).

The HL-A system (Human leukocyte locus A) is considered to be one of the major histocompatibility systems in man. It exhibits a high degree of polymorphism and is one of the most complex genetic systems studied. The HL-A antigens are determined by genes at the HL-A region on a pair of autosomal chromosomes. This region is divided into two loci (sub-loci), each expressing antigens determined by one of a series of mutually exclusive alleles. Any individual will therefore possess two antigens from each series, making a total of four. The HL-A system has come into prominence in the field of organ transplantation, but more recently, interesting relationships between the frequency of certain HL-A antigens and disease have been described.

Two recent studies of HL-A phenotypes in patients with ACD have shown a significantly increased incidence of HL-A8 in this condition when compared with control populations (Stokes *et al.*, 1972; Falchuk, Rogentine & Strober, 1972), thus we felt that a similar study of patients with DH would help to clarify the relationship of the two conditions. Prior to publication of our findings, a study of HL-A antigens in DH had been reported by Katz *et al.* (1972), who found an increased incidence of HL-A8 in this condition.

TABLE 1. The incidence of HLA antigens in healthy controls and patients with dermatitis herpetiformis

HL-A antigen	Controls (n = 175)		Dermatitis herpetiformis (n = 35)		Chi-square >1 (Yates' corrected)	*P* (after Bonferroni correction)
	No.	%	No.	%		
1	61	34·8	19	54·3	3·88	NS
2	75	42·8	10	28·6	1·91	NS
3	44	25·1	9	25·7	—	NS
9	28	16·0	6	17·1	—	NS
10	15	8·7	4	11·4	—	NS
11	29	16·6	4	11·4	—	NS
W19	23	13·2	3	8·6	—	NS
5	12	6·9	1	2·9	—	NS
7 Complex*	82	46·9	17	48·6	—	NS
8	58	33·1	21	60·0	7·86	0·05
12	53	30·3	9	25·7	—	NS
13	7	4·0	0	0·0	—	NS
W15	15	8·7	0	0·0	—	NS
W17	12	6·9	2	5·7	—	NS
W14	13	7·5	1	2·9	—	NS
1+8	46	26·3	14	40	2·47	NS

* Includes HL-A7, W10, W22, W27. NS, not significant.

MATERIALS AND METHODS

Patients

Thirty-five unrelated patients with DH were investigated; the diagnosis having been made on the clinical and histological features of the rash, its clearance with dapsone or sulphapyridine, and relapse on withdrawal of the drug. At the time of the study, twenty-one patients were receiving dapsone or sulphapyridine alone, and eleven were receiving a gluten-free diet (GFD) plus dapsone or sulphapyridine. Three were on GFD alone. Jejunal biopsies were performed on thirty-one of these patients.

Controls

175 healthy blood donors in Southern Scotland comprised the control group. The HL-A antigen frequency in this group did not differ significantly from a control group in the UK described by Stokes *et al.* (1972). Potential cadaver donors were not included as controls in our study, because

the frequency of certain antigens (notably HL-A1 and 8) was considerably lower than that found in the healthy normals (White, da Costa & Morton, 1972).

Methods

Lymphocytes were separated from defibrinated blood by centrifugation in a Ficoll–Triosil gradient (Bøyum, 1968). Typing for HL-A antigen was carried out using a modified Terasaki lymphocytetoxicity technique with glass typing trays (Terasaki & McClelland, 1964).

At least three antisera were used to determine each HL-A antigen. Reproducibility of the typing technique used was determined by repeated typings of controls, and found to be at least 95% for the individual serological reactors, and to approach 100% for the HL-A phenotypes tested. Jejunal biopsy was carried out using the Crosby capsule positioned in the proximal jejunum under X-ray control. Only one biopsy was performed on each patient.

RESULTS

The frequencies of HL-A antigens in both control and DH groups are shown in Table 1. The occurrence of HL-A8 in the DH group is significantly greater when using the chi-square test with Yates' correction ($P<0{\cdot}005$). When adjusted for Bonferroni inequality (Miller, 1966) this level is still significant. Out of thirty-one jujenal biopsies performed prior to GFD, twenty-three were abnormal (partial or subtotal villous atrophy) and 8 were normal.

When the results of the twenty-three patients with abnormal jejunal biopsies were compared with the controls, the frequency of HL-A8 in this group was slightly higher (65·5%). The incidence of HL-A8 in the eight cases with normal biopsies was 50%. This difference is not significant.

DISCUSSION

This study provides further evidence of a similarity between DH and ACD. The incidence of HL-A8 in the DH patients was 60%, as compared with 33·1% in the normal controls. These results are similar to those published in a recent paper (Katz *et al.*, 1972), where 58% of DH patients had HL-A8, as compared with 24% of controls. The association of HL-A8 with DH is not as strong as that reported for ACD by Stokes *et al.* (1972), who found that 88% of ACD patients had HL-A8, and by Falchuk *et al.* (1972), who showed an 87% incidence. Like Katz *et al.* (1972), we found an increased incidence of HL-A1, though the difference from the control series was not significant.

When only those patients with abnormal single jejunal biopsies prior to GFD are considered, the incidence of HL-A8 is higher; but does not differ significantly enough from the group with normal biopsies to indicate a greater susceptibility to the bowel lesion in those patients carrying this antigen, as suggested by Katz *et al.* (1972). However, even these results ought to be interpreted with caution in view of investigations of the jejunal mucosa in DH by Brow *et al.* (1971) using a multiple biopsy technique, in which they found that jejunal changes in this condition were present in almost 100% of patients, and not in 67% as previously supposed.

Katz *et al.* (1972) also suggested that the genes controlling HL-A antigens may be linked with, or identical with, 'immune response' genes, and that the immunological phenomena reported in DH could be under the control of an 'immune response' gene related in some way to the HL-A8 histocompatibility gene. HL-A8 has indeed been associated with several diseases known to involve derangement of the immune system, e.g. childhood asthma, acute lymphatic leukaemia (Thorsby, Engeset & Lie, 1971), Hodgkin's disease (Falk & Osoba, 1971), susceptibility to transplant rejection (Kissmeyer Nielsen, 1972), systemic lupus erythematosus (Grumet *et al.*, 1971), and chronic active hepatitis (Mackay & Morris, 1972). With this in mind, we studied circulating auto-immune antibodies in our DH series, including thyroid and gastric antibodies, and antinuclear factors. It was found that ten of twenty-one patients with HL-A8 had auto-antibodies, as opposed to seven of fourteen patients who did not have this antigen. There was thus no significant difference between the two groups.

ACKNOWLEDGMENTS

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The proliferation of cells in the dermal infiltrate of lichen planus lesions

J.-M. LACHAPELLE AND M. DE LA BRASSINNE

Unit for Occupational Dermatoses, Louvain University,
4, avenue Chapelle-aux-Champs, 1200 Brussels, Belgium

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SUMMARY

The proliferation of mononuclear cells infiltrating the dermis in lichen planus lesions was studied in five untreated patients. A biopsy of a lesion was made in each patient. Thin slices of the specimens were incubated for 60 min in the presence of ^{3}H thymidine (^{3}HT), fixed and radioautographed. The mean value of the ^{3}HT labelling index (±SE) was $2{\cdot}87 \pm 0{\cdot}14\%$. This labelling index (LI) is relatively high; it is not statistically different from the LI encountered in the dermal infiltrate of allergic contact dermatitis ($P > 0{\cdot}45$), but is three to four times higher than in psoriasis or irritant dermatitis.

The proliferative cells are most probably lymphoblasts. A biopsy of another lichen planus lesion was made in two of the five patients 60 min after a local injection of 10 μCi of ^{3}HT. Imprints of lesions were treated histochemically for Naphthol AS-D acetate esterase(s) identification, a useful method for distinguishing monocytes (many cytoplasmic granules) and lymphocytes (few if any granules). The imprints were then radioautographed. No ^{3}HT-labelled cells contained more than one or two cytoplasmic granules, which suggests that they are of lymphocytic origin.

The biological significance of this lymphoblastic proliferation still remains hypothetical.

To our knowledge, there is no report in the literature concerning the proliferative activity of the cells infiltrating the dermis in lichen planus lesions.

The purpose of the present work is to characterize that activity by means of single pulse label with ^{3}H thymidine in order to identify cells in the phase of DNA synthesis.

The possible significance of our results will be discussed in relation to some recent data about lichen planus.

MATERIALS AND METHODS

This investigation was made in five patients with untreated lichen planus. A 4 mm punch biopsy of a papule of recent onset was obtained in each case. The biopsies were cut into very thin slices (<1 mm), which were incubated at 37°C for 1 h in a culture medium containing 2 μCi of 6-^{3}H thymidine (^{3}HT; specific activity 10 Ci/mmol; concentration 1 mCi/ml; Centre d'Energie nucléaire, Mol, Belgium) according to the technique of Lachapelle & Gillman (1969). They were then fixed in 4% buffered formalin for 24 h, paraffin embedded, cut, and processed for light microscopy radioautography (dipping technique of Kopriwa & Leblond (1962) using the Kodak NTB2 emulsion; exposure time, 14 days). The sections were stained with haematoxylin–eosin.

In each specimen, 2000 nuclei of the 'round' cells of the dermal infiltrate were counted. The ^{3}HT nuclear labelling index [(number of labelled cell nuclei/total number of counted cell nuclei) × 100] was determined in all specimens.

In addition, an intradermal injection of 10 μCi of ^{3}HT, diluted in 0·1 ml normal saline, was performed in another lichen planus papule in two of the five patients. A 4 mm punch biopsy of the injected site was obtained 1 h later. Imprints were prepared from these biopsies, according to the technique of Degos & Ossipowski (1957). Some of the air-dried imprints were fixed in methanol for 3 min, radioautographed (exposure time, 8 h) and stained with May-Grünwald-Giemsa (MGG); the others were treated histochemically for Naphthol AS-D acetate esterase(s) identification (according to the method of Burstone (1957) using the Naphthol AS-D acetate substrate in 0·2 M tris-maleate buffer at pH 6·5 with Fast Red Salt R.C. as an azo dye), fixed in 4% buffered formalin for 10 min, radioautographed (exposure time, 8 h), and stained with haematoxylin.

RESULTS

Histological findings

In the five cases, the histological lesions were typical of 'established' lichen planus, according to the criteria of Black & Wilson Jones (1972), i.e. hyperkeratosis, epidermal thickening, saw-tooth appearence of the rete ridges with a band-like infiltrate of mononuclear cells.

Colloid bodies were present in all cases. Necrosis of some basal epidermal cells with some inflammatory 'round' cells between the lower epidermal cells was conspicuous in two biopsies, but less obvious in the three others.

Melanin-laden macrophages were numerous in the upper dermis of most lesions.

Cytological and cytochemical findings

A detailed study of MGG stained imprints showed a mixture of round and more elongated cells; moreover, melanin-laden macrophages were easily recognizable.

The cytochemical detection of Naphthol AS-D acetate esterase(s) gave the following results: most of the 'round' cells were negative or feebly positive (one or two granules in their cytoplasm) and, therefore, were presumably of lymphocytic origin. Nevertheless, a few of them were loaded with granules, which suggested that they were monocytes. Most of the elongated cells were heavily positive and, thus, probably represented the histiocytic component of the infiltrate.

Histo- and cyto-radioautographic findings

In the lesions of lichen planus, the ^{3}HT labelling of epidermis was limited to a small number of basal cells, which were very often sickle-shaped (Fig. 1). This confirms the recent observations of Marks, Black & Wilson Jones (1973).

A relatively large number of cells in the dermal infiltrate were involved in the cell mitotic cycle. These labelled cells were distributed throughout the band-like infiltrate (Fig. 1). The mean nuclear labelling index of 'round' cells was $2{\cdot}87 \pm 0{\cdot}14\%$. A few elongated cells incorporated ^{3}HT, but none of the melanin-laden macrophages could be found in the S-phase. Labelled endothelial cells of blood capillaries could exceptionally be encountered.

Radioautographs of imprints treated histochemically for Naphthol AS-D acetate esterase(s) identification, showed that all the ^{3}HT labelled 'round' cells contained very few, if any, cytoplasmic (specific for esterase) granules. Round cells, which were heavily positive for Naphthol AS-D acetate esterase(s),

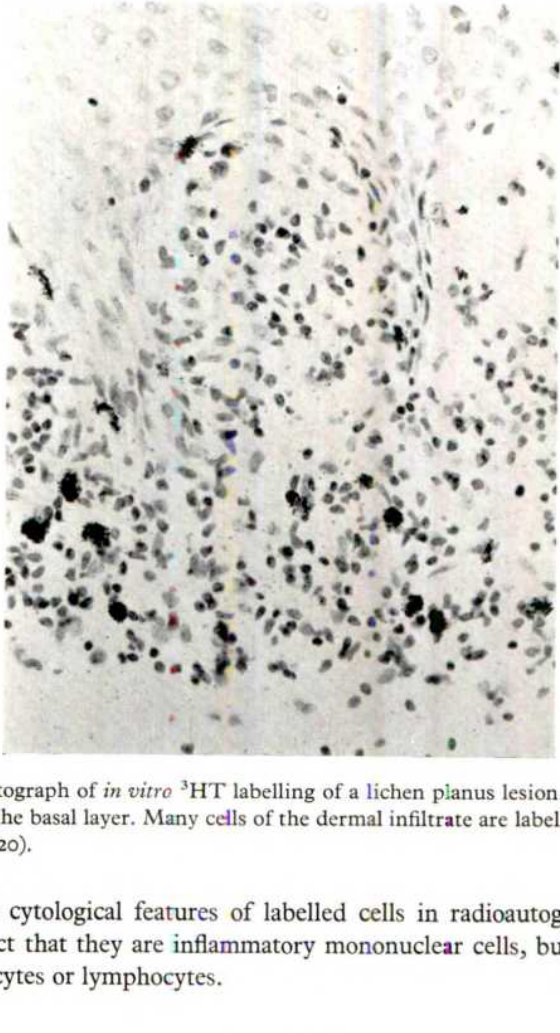

FIGURE 1. Radioautograph of *in vitro* ^{3}HT labelling of a lichen planus lesion. Epidermal labelled cells are limited to the basal layer. Many cells of the dermal infiltrate are labelled. (Exposure time, 14 days; H & E, × 220).

were not labelled. The cytological features of labelled cells in radioautographs of MGG stained imprints confirm the fact that they are inflammatory mononuclear cells, but do not permit classification of them as monocytes or lymphocytes.

DISCUSSION

An interesting finding of the present study concerns the relatively high ^{3}HT LI of 'round' cells infiltrating the dermis in lichen planus. This becomes particularly obvious, when compared (Fig. 2) with the LI of the dermal infiltrate in other skin conditions, such as irritant and allergic contact dermatitis (Lachapelle, 1972), or psoriasis (Lachapelle & de la Brassinne, 1972). The proliferative activity of the cells of the infiltrate is three to four times higher in lichen planus and allergic contact dermatitis than it is in psoriasis or irritant dermatitis. There is no statistically significant difference by the Student's *t*-test ($t = 0{\cdot}103$; $P > 0{\cdot}45$) between allergic contact dermatitis and lichen planus.

A careful examination of serial sections showed that labelled 'round' cells were uniformly distributed throughout the infiltrate, and not preferentially around the blood capillaries.

It has been repeatedly shown in the haematological literature (Wachstein & Wolf, 1958; Braunstein, 1959; Braunsteiner & Schmalzl, 1968; Daniel *et al.*, 1971) that the identification of Naphthol AS-D acetate esterase(s) activity in white blood cells is a good method for distinguishing between lymphocytes (negative or feebly positive) and monocytes (heavily positive). Moreover, many authors have

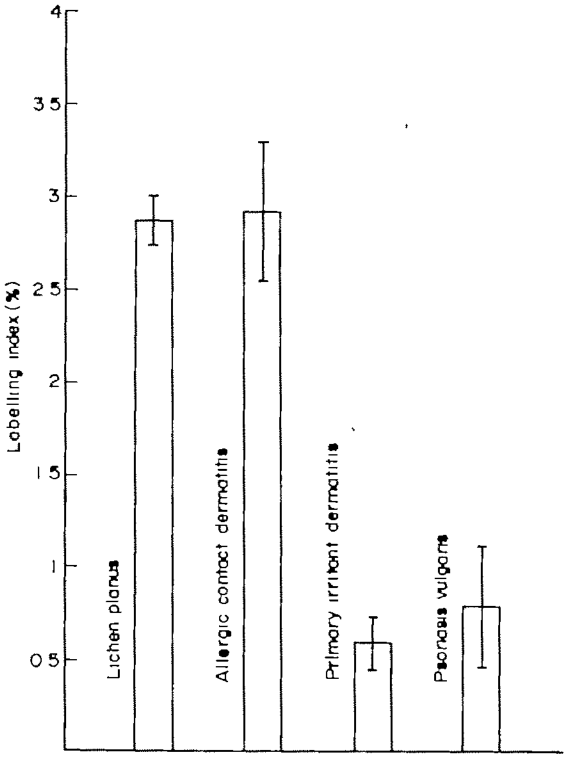

FIGURE 2. ^{3}HT labelling index in dermal infiltrate in lichen planus, allergic contact dermatitis, primary irritant dermatitis and psoriasis.

used the Naphthol AS-D acetate technique to characterize the nature of the cells infiltrating the dermis in different pathological skin conditions, such as allergic contact dermatitis (Bandmann, 1967), lichen planus (Berger, Hundeiker & Engelhardt, 1969) or psoriasis (Hundeiker, 1970). The infiltrate was shown in all conditions to be a mixture of lymphocytes and monocytes (some of them transformed locally into histiocytes).

By combining cytochemical and radioautographic techniques on the same imprints in our cases of lichen planus, we have been able to show that the 'round' cells which are proliferating in the infiltrate are lymphoblasts. Indeed ^{3}HT labelled cells are negative (or very feebly positive) for Naphthol AS-D acetate esterase(s). The presence of these lymphoblasts still remains unexplained. It is not known whether they synthesize DNA before reaching the skin or begin to replicate in the dermis itself (as a response to an hypothetical epidermal 'trigger' factor from degenerative cells). The 'blast' transformation could represent either a nonspecific phenomenon or an immunological response. The results of the experiments by Sarkany & Gaylarde (1972) on *in vitro* lymphocyte transformation in lichen planus are too preliminary to support evidence in favour of an auto-immune reaction.

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In vitro lymphocyte studies in chronic polymorphic light eruption

E.J.RAFFLE, T.M.MACLEOD AND F.HUTCHINSON*

Department of Dermatology, University of Dundee and *Regional Physics Department

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SUMMARY

Lymphocytes suspended in autologous sera from seven subjects with chronic polymorphic light eruption were irradiated with UV radiation corresponding to the peak of their action spectra, and lymphocyte transformation and migration inhibition studies were subsequently carried out. The results obtained do not support a concept of photoallergy, but an increased thymidine uptake ratio was obtained in one subject, the only one who had shown clinical involvement of covered skin sites in addition to light exposed ones.

In patients with chronic polymorphic light eruption (CPLE) varying admixtures of prurigo, erythema, oedema, and lichenification are seen on exposed skin, and also at times on covered areas, with maximum affection in the sunshine months (March–October). The condition occurs mainly in females and appears for the first time usually before adult life. Sufferers from this condition show an abnormal response to light with action spectra both within and above the sunburn range (290–320 nm) (Frain-Bell, Mackenzie & Witham, 1969). The underlying biochemical mechanism of the condition is by no means clear. It has been suggested that as the result of an inflammatory response to a particular wavelength of light metabolites are formed, which in some individuals are antigenic and provoke a cell mediated reaction (Epstein, 1942; Epstein, 1971). The concept of a delayed hypersensitivity response is supported by the histological findings of a dense perivascular lymphocytic infiltrate, the incubation period of several hours or several days, the flare of previously involved areas with exposure of distant sites, and the fact that after several years patients seem to lose all resistance to sunlight and have eruptions throughout the winter as well as the summer months. If a lymphocyte sensitizing metabolite is responsible for initiating a cell mediated response and subsequent skin damage, one might expect that lymphocytes from CPLE subjects would behave differently to those from normal subjects in an *in vitro* test system where they can be irradiated and their responses monitored. Both the lymphocyte transformation test and the migration inhibition test have proved valuable in the study of delayed hypersensitivity.

We describe here our investigations using these tests in an investigation of CPLE.

MATERIALS AND METHODS

Lymphocyte transformation tests (LTT)

Seven subjects with CPLE were studied; one of these (Case 6) had previously also shown an urticarial

TABLE 1. Thymidine uptake tests in irradiated samples from CPLE patients and control subjects (results expressed as d.p.m. ± SD)

Case	Diagnosis	Irradiation (λ nm)	Lymphocytes (Count/mm³)	Control culture (d.p.m.)	PHA Culture	UV irradiated cultures					
						d.p.m.	Dose mJ/mm²	d.p.m.	Dose mJ/mm²	d.p.m.	Dose mJ/mm²
1	CPLE	305±15	6068	18±2	3334±1027	6±1	0·15	8±1	1·54	6±1	4·62
2	CPLE	305±15	4095	32±15	7856±1644	13±2	0·15	12±1	1·50	7±1	4·50
3	CPLE	305±15	5785	27±1	446±79	39±18	0·0165	56±19	0·165	18±1	1·65
4	CPLE	305±15	5655	17±5	5922±1718	8±1	0·50	18±5	2·50	15±5	5·00
5	CPLE	295±15	8117	721±63	4117±1368	735±80	0·012	695±86	0·12	732±72	1·26
6	CPLE	400±30	6365	16±1	3328±684	23±5	0·90	22±1	4·50	10±1	9·00
7	CPLE	320±15	6820	27±7	ND*	59±34	0·0158	73±34	0·092	78±34	1·01
8	Normal	305±15	3886	13±1	11223±2541	5±1	0·156	8±3	1·56	24±11	4·65
9	Normal	305+15	4824	54±16	9329±639	16±4	0·156	49±11	1·56	10±1	4·65

* ND, not done.

response to sunlight exposure. All of them had been extensively investigated by phototesting (Frain-Bell *et al.*, 1973). They comprised six females and one male (Case 5): two control subjects were also studied, one male, one female.

The technique of lymphocyte transformation testing which was used has been previously described (Hutchinson, Raffle & Macleod, 1972). 0·5 ml aliquots of lymphocytes obtained by gravity sedimentation of heparinized blood for 1 h were suspended in the patient's own serum ($2–4 \times 10^{-6}$ lymphocytes per ml). They were then irradiated in open polystyrene vials (2·5 cm diameter) under aseptic conditions using three different doses at wavebands corresponding to the peak of the phototest action spectrum of the patient (Table 1). The aliquot volume and polystyrene vial diameter were chosen to give a sample thickness < 1 mm, thus ensuring minimum attenuation by the irradiated sample. The light source used was a 1·6 kW xenon arc lamp coupled to a Bausch and Lomb (33-86-48) grating monochromator. Output was measured by means of a Hilger Watt thermopile and Solartron digital voltmeter. A WG345 Schott filter was used at 400 nm to eliminate scatter and second order spectra. The cell suspensions were irradiated at the chosen wavelength peaks and details of the half bandpass (BP) are listed in Table 1. At 305 nm the bandpass was rectangular, but triangular at all other bandpasses. The doses were chosen to correlate with the physiological range used for testing *in vivo*. The lowest dose range used correlated approximately with the patient's phototest MED.

Variation of UV output over the irradiated area was ±15%. After irradiation the 0·5 ml aliquots of lymphocyte suspensions were transferred to culture tubes with 1·5 ml medium 199 so that the final cell concentration was $0{\cdot}5–1{\cdot}0 \times 10^{-6}$/ml culture. Each sample was set up in quadruplicate and the cultures were incubated at 37°C for 4 days. Phytohaemagglutinin (PHA) cultures of unirradiated lymphocytes were carried out in all subjects and of irradiated lymphocytes in some subjects. The PHA transformation tests were used as an index of the immunological competence of the patients lymphocytes. DNA synthesis was measured using a 5 h pulse of 0·1 μCi 2^{14}C thymidine (58 mCi/mmol). Harvesting procedures were idential to those described previously (Hutchinson *et al.*, 1972). Viability checks using Trypan Blue were also carried out on each sample.

Tests for migration inhibition factor (MIF)

The method described by Federlin *et al.* (1971) for peripheral leukocyte migration was used in five female cases of CPLE. Lymphocytes from gravity sedimented heparinized peripheral venous blood were concentrated by centrifugation at 150 g for 5 min, washed three times with medium 199, and resuspended in a concentration of 70×10^{-6}/ml. Capillary tubes containing 20 μl of this suspension were centrifuged at 600 **g** for 10 min and the tubes were cut at the cell/medium interface. The cell columns were mounted on silicone dabs in wells of a Sterilin plate containing medium 199 plus 25% of patients' own serum, either unirradiated or irradiated with the intermediate dose and wavelength employed in the LTT. Two capillary tubes were mounted per well and the tests were carried out in triplicate. After 18 h incubation the zones of cell migration were charted by projection microscope and measured by planimetry. The standard deviation of the measurements was better than 10%.

RESULTS

The results of the lymphocyte transformation testing are shown in Table 1, and the thymidine uptake ratios (TUR) shown in Fig. 1. An increase in TUR occurred in one case only (Case 7). The MIF tests (Table 2) did not show any significant difference from the controls (i.e. >20% inhibition). It would have been ideal to carry out MIF tests on all the subjects in whom LTTs were performed, but this was not practicable. Viability tests showed that the fraction of cells surviving relative to the

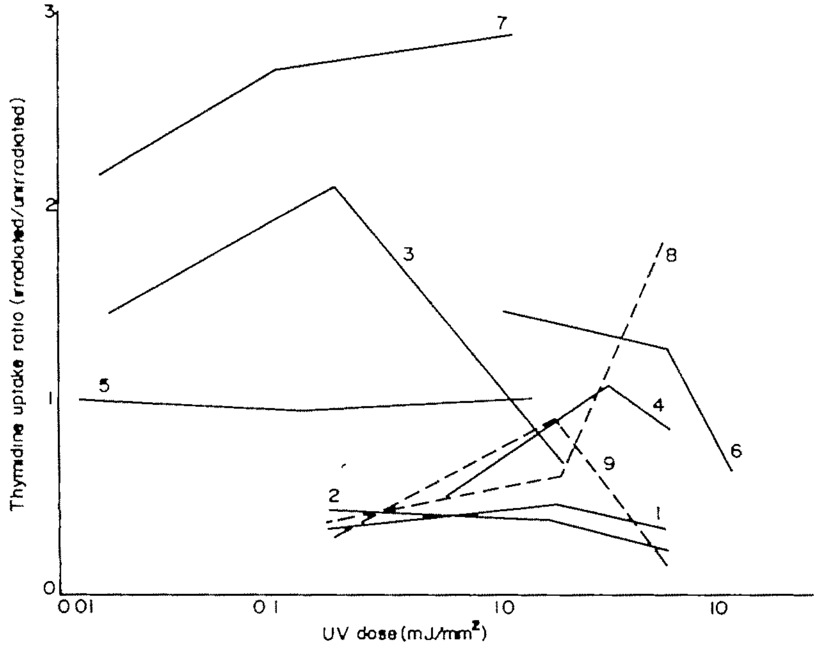

FIGURE 1. Results of LTT after UV irradiation in seven patients and two normal controls. ———, Patients; – – – – – –, controls.

TABLE 2. MIF testing. Results on cells suspended in irradiated serum from CPLE patients and controls

Case	MIF ratio $\frac{\text{(irradiated)}}{\text{(unirradiated)}}$
1	1·10
2	0·86
3	1·11
8	1·03
9	1·05
10	0·83
11	1·03

Irradiation dose = 1·5 mJ/mm²; λ = 305 ± 15 nm.

unirradiated samples was 0.89 at 1·0 mJ/mm², 0·69 at 5·0 mJ/mm², and 0·61 at 10·0 mJ/mm². The PHA response was normal in all subjects, except subject 3, where it was low. Irradiated samples were shown to be capable of responding to PHA, and the responses were of the same order as those of the unirradiated samples.

DISCUSSION

Our results indicate that irradiation of serum and lymphocytes from subjects with CPLE based on the action spectrum of the individual patient produces no significant effect on DNA synthesis when the lymphocytes are cultured in the standard manner, nor is there any release of serum migratory inhibition factor after irradiation. However, one subject (Case 7) did show an increased TUR and this subject differed from the others in that she was the only one in whom there had been involvement of covered sites in addition to light exposed skin sites. The increase cannot be caused by DNA repair mechanism, since unscheduled incorporation is complete within 6 h post-irradiation, and also the percentage uptake relative to the normal DNA synthesis is small (Evans & Norman 1968; Cleaver, 1970).

These findings differ from those reported by Horkay & Meszaros (1971). They found that UV radiation increased the TUR of cultured lymphocytes from CPLE patients. Since no indication is given in their paper of the errors involved in their measurements these findings are not of themselves conclusive, but they were able to report increased transformation based on morphological assessment also with UV. However, in the only case in which both cell morphology and thymidine incorporation tests were carried out, the results were at variance.

Possible explanations for the discrepancies between our results and those of Horkay & Meszaros (1971) could lie with the irradiation conditions used or with the clinical state of the patients subjected to these *in vitro* tests. The irradiation conditions used in our experiments differed from those of Horkay & Meszaros. Whereas these workers used two broad bands, > 320 nm and > 200 nm, it seemed more logical to us to irradiate the specimens at the peak of the action spectrum found for each individual patient.

In experiments such as this, there are many important factors which can influence the result. The lymphocyte test system has been isolated *in vitro*, and skin factor(s) which may be present at the site of clinical signs and symptoms, or produced by exposure to light are not included in the system under test. Moreover, if a photoallergen is involved, the UV waveband and/or the dose range used may be inappropriate for its production or could, if the dose were too high, impair the response which was being sought.

The duration of the culture (4 days) was the same as that used by Horkay and Meszaros. It may be argued that, since the response to certain allergens reaches a peak at 6 days, a 4 day exposure is too short, but if the present test system had included an allergen then even the 4 day culture would be sufficient to demonstrate a trend. PHA-induced lymphocyte transformation provides a direct measure of T-cell function and thus indicates competence of the cell-mediated immune system. Irradiated lymphocytes are capable of responding to PHA stimulation and hence the dose range used was not 'toxic' to the allergenic stimulation. This would indicate that except for Case 7, UV irradiation of lymphocytes from CPLE patients does not result in their transformation. It is unfortunate that it was not feasible to carry out MIF tests on the serum from this particular patient, but the negative results of the sera tested in the way described above suggest that the action of UV on patients' sera does not release an allergen capable of inducing the liberation of leukocyte inhibiting factors from their own leukocytes. One might speculate that because Case 7 had a positive LTT, a cell-mediated mechanism could underly the state of affairs when dissemination of the condition to sites other than light exposed ones occurs; such a single result is more likely to be an experimental artifact.

Further studies along two lines could follow from these findings. One would be to assess the influence of varying wavebands and/or radiation dose in the lymphocyte test system; the other to include skin or skin extracts in the biological system. The latter would appear to offer more promise of success.

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Fabry's disease: normal α-galactosidase activity and urinary-sediment glycosphingolipid levels in two obligate heterozygotes

JOSÉ LUIS AVILA, JACINTO CONVIT AND
GLADYS VELAZQUEZ-AVILA

Department of Dermatology, 'José Vargas' School of Medicine,
Central University of Venezuela, Caracas, Venezuela

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SUMMARY

A clinical and biochemical study of twelve persons in the same family, including two hemizygous and one typical heterozygous patients, for Fabry's disease is reported. Two obligate heterozygous females—daughters of the index case—lacked any of the clinical manifestations found in typical heterozygous carriers, having also normal α-galactosidase activity in peripheral leukocytes and normal urinary excretion of trihexosylceramide and digalactosylceramide. Blood group serology was determined to exclude illegitimacy. The validity of these findings and some possible explanations are discussed.

Fabry's disease (angiokeratoma corporis diffusum) is characterized by the deposit of a glycolipid within arterial smooth muscle, myocardium, kidney, bone marrow, reticulo-endothelial system and various components of the nervous system (Colley *et al.*, 1958; Rahman & Lindenberg, 1963; Frost, Tanaka & Spaeth, 1966; Sagebiel & Parker, 1968). Typically, the disease is characterized in the affected males by its dermatological manifestations: multiple small purplish macules and papules in the umbilical region, scrotum and other areas; and systemically by renal lesions (lesions of the glomerular epithelium and proximal and distal tubules) with proteinuria, cardiovascular disease, ocular abnormalities (corneal opacities, cataracts and dilatation and tortuosity of retinal and conjunctival vessels), attacks of fever and burning pain in the extremities, and dysfunction of the central nervous system and gastrointestinal tract.

The affected male's life expectancy is reduced, and death usually occurs between the ages of 40 and 50 as a result of renal failure (Sweeley & Klionsky, 1966).

The abnormal gene responsible for this glycosphingolipidosis is sex linked, with occasional and variable penetrance in the heterozygous female and full penetrance in the homozygous female. Heterozygous women have limited clinical manifestations (Wallace, 1958), which are mainly corneal dystrophy (80–90%) (von Gemmingen, Kierland & Opitz, 1965), and in a few cases an inconspicuous rash (Wise, Wallace & Jellinek, 1961), attacks of burning pain in the extremities (Jeffries &

Requests for reprints to Dr José Luis Avila, Instituto Nacional de Dermatología, Apartado Postal 4043 (Carmelitas), Caracas, Venezuela.

Barrett, 1963), and the characteristic renal glomerular lesion (Rahman *et al.*, 1961). Rarely, a female may be afflicted as severely as the affected male (Opitz *et al.*, 1965). Recently, it has been shown that the lipids accumulated in renal tissue obtained at autopsy are mainly two: ceramide trihexoside and ceramide dihexoside, the former being predominant (Sweeley & Klionsky, 1963). On the other hand, Brady *et al.* (1967) have found a deficiency of ceramide trihexosidase activity in the intestinal mucosa of two hemizygous males with Fabry's disease, while a heterozygous female showed reduced enzymatic activities. These enzymatic studies are consistent with an X-linked mode of inheritance and establish a connection between the enzymatic deficiency and the accumulation of gal-gal-gl-cer in these patients.

Recently, from NMR and IR spectra, and by using α- and β-galactosidases, it was concluded that terminal galactose in the ceramide trihexoside has an α configuration (Hakomori *et al.*, 1971). Consistent with this finding, Kint (1970), using peripheral blood leukocytes, has showed a very marked deficiency of α-galactosidase activity in patients hemizygous for Fabry's disease, and intermediate values of enzymatic activity in heterozygous females. These results have been confirmed also by Romeo & Migeon (1970), who have also shown that fibroblasts from the mother and sister of the propositus had two distinct clonal populations, one with normal α-galactosidase activity, the other being enzyme deficient, providing evidence of genetic inactivation of one of two X chromosomes at this locus in female somatic cells.

In this paper we wish to describe the clinical and biochemical findings obtained in two obligate heterozygous patients for Fabry's disease. These two 4- and 7-year-old sisters had no clinical ophthalmic or cutaneous manifestations of the disease. They had normal amounts of trihexosyl and digalactosylceramides in the urine, and also normal α-galactosidase activity in peripheral polymorphonuclear leukocytes.

CASE REPORTS

Case 1 (*III*, 2, *index case*)

A 45-year-old man had noticed for 20 years the appearance of bluish-red excrescences in the skin, first on his hips, then on the tips of his fingers and later on the scrotum, umbilical region and in both flanks.

For about 15 years he had experienced paroxysmal painful episodes in hands and feet, lasting several hours and improving with analgesics. For the last 3 years he had complained of crises of abdominal pain, accompanied by chills, generalized tremors, vomiting and fever of 39°C; these crises disappeared with antispasmodics and they generally recurred every 2 or 3 months, simultaneously with intense albuminuria. The albuminuria subsided when the abdominal pain disappeared. He also reported that for the last 5 years he had had oedema of both legs in the afternoons, increasing on standing or even sitting. A physical examination revealed the following data: weight 46·5 kg; height 1·63 m; blood pressure, 125/70; pulse 65, and regular. Upon dermatological examination an eruption was seen, consisting of dark purple micropapules, varying in size between 0·5 and 3 mm, localized primarily in the hypogastrium, scrotum, flanks, hips and gluteal regions, dorsal and lateral aspects of both thighs, and in smaller numbers on the medial aspects of both arms and forearms, on the palms and backs of both hands and in the thoraco-abdominal region. These lesions did not disappear with pressure (Fig. 1).

The buccal and sublingual mucosa was speckled with angiokeratotic spots. The rest of the physical examination revealed varicosities of medium calibre of the internal saphenous vein of both legs, and oedema and hyperkeratotic, desquamating lesions on the lower third of both legs.

The results of the laboratory tests are presented in Table 1. Renal investigation revealed moderate proteinuria, normal urea and creatinine clearance and negative urine culture. ECG changes were

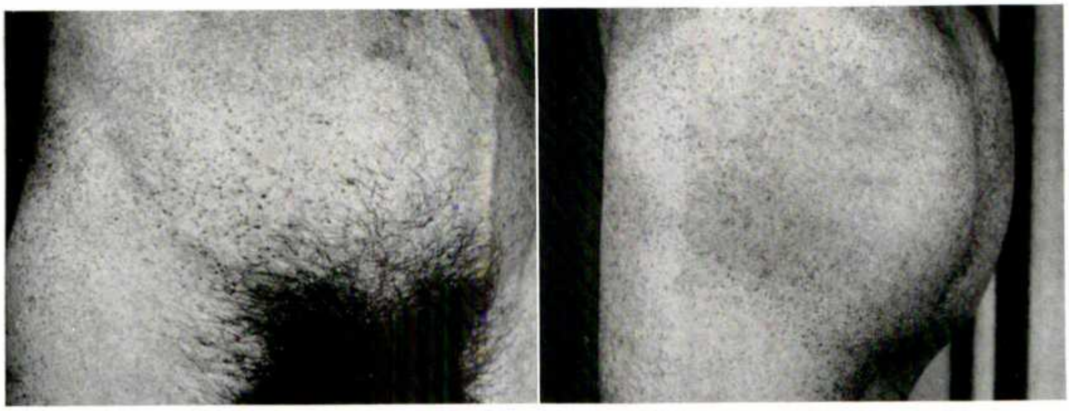

FIGURE 1. Skin lesions found in a patient hemizygous for Fabry's disease (index case). Note the presence of multiple isolated fine micropapules.

TABLE 1. Results of selected laboratory and other tests (Cases 1 and 2)

Determinations	Case 1	Case 2
Blood		
Haemoglobin (g/100 ml)	13·2	12·6
Haematocrit (%)	42	41
White blood cell count (per mm^3)	6750	7360
Platelet count (per mm^3)	232,000	283,000
Sedimentation rate (mm/h)	22	12
Serum		
Proteins (g/100 ml)		
Total	6·80	7·10
Albumin	3.77	3·89
Alpha$_1$-globulin	0·27	0·32
Alpha$_2$-globulin	0·36	0·52
Beta-globulin	0·90	0·91
Gamma-globulin	1·50	1·46
Uric acid (mg/100 ml)	3·90	4·30
Creatinine (mg/100 ml)	0·70	0·90
Urea (mg/100 ml)	14·00	26·00
Glucose, fasting (mg/100 ml)	84·00	92·00
Total cholesterol (mg/100 ml)	206·00	314·00
Triglycerides (mg/100 ml)	115·00	150·00
Total lipids (mg/100 ml)	650·00	723·00
Urine		
Proteinuria (mg/100 ml)	440·00	321·00
Density (g/cm^3)	1·010	1·023
Urea clearance (ml)	64	53
Creatinine clearance (ml)	92	83

compatible with chronic coronary insufficiency and enlargement of the left ventricle; radiological examination of the chest revealed cardiac diameters within normal limits. An ophthalmological study revealed bilateral corticonuclear opacities, marked tortuosity of the conjunctival and retinal veins (corkscrew shaped); the retina was of normal appearance. The bone marrow had abundant cells with multiple intracytoplasmic vacuoles filled with lipids (stained with Sudan III). The gastrointestinal tract was normal, except for internal haemorrhoids.

Case 2 (III, 7)

A 58-year-old man, double first cousin of the index case, noted the appearance, when he was about 19, of asymptomatic angiokeratotic lesions, first in the gluteal region and on the thighs and later also in the inguinal regions, on the scrotum and on the back. For about 15 years he had suffered each year three or four paroxysms of burning pain of the hands and feet, crises of abdominal pain of short duration, fever of 39°C and oedema of both legs.

Physical examination revealed findings similar to those described for the index case. The laboratory tests showed minimal proteinuria, with normal endogenous urea and creatinine clearance.

Case 3 (III, 5)

A 50-year old woman, sister of the index case had had no symptoms. Physical examination was within normal limits except for bilateral corneal opacities on slit-lamp examination. Clinical laboratory tests were normal.

Case 4 (IV, 2)

This 7-year-old girl, the firstborn child of the index case and a healthy woman, was delivered after a full-term, normal pregnancy and weighed 3 kg. Apart from childhood exanthematic fevers her clinical history was negative. Physical examination was within normal limits. Careful examination of skin and eyes revealed no abnormalities. Sex chromatin analysis was positive.

Case 5 (IV, 1)

This 4-year-old sister of Case 4 showed on physical examination no abnormalities either in the skin or the eyes. Clinical laboratory tests were normal. Sex chromatin analysis was positive.

The pedigree of the patients is shown in Fig. 2.

MATERIAL AND METHODS

Enzymatic studies

For these studies we used polymorphonuclear leukocytes obtained from peripheral blood. Leukocytes were separated by the method of Avila & Convit (1970), which, in brief, consists of sedimentation of the white blood cells in dextran 2%, and two hypotonic shocks to decrease red cell contamination. Cells were lysed using 0·34 M sucrose and sonication (because 100 U heparin/ml induced a 66% inhibition in α-galactosidase activity, Fig. 3b). The enzymes studied were: α-galactosidase, N-acetyl-β-glucosaminidase, β-glucuronidase and β-galactosidase. The methods used for the determination of the various enzymatic activities have already been published (Avila & Convit, 1970) except for that for α-galactosidase, which was assayed by the method of Kint (1970) with slight modifications: incubation was in acetate buffer at pH 5·0 rather than pH 4·5, and *O*-nitrophenyl-α-D-galactopyranoside was used instead of *p*-nitrophenyl-α-D-galactopyranoside, and the final volume was 800 μl. The enzymatic tests were carried out at 37°C for all the enzymes, in duplicate, each time with two different volumes of the enzymes tested.

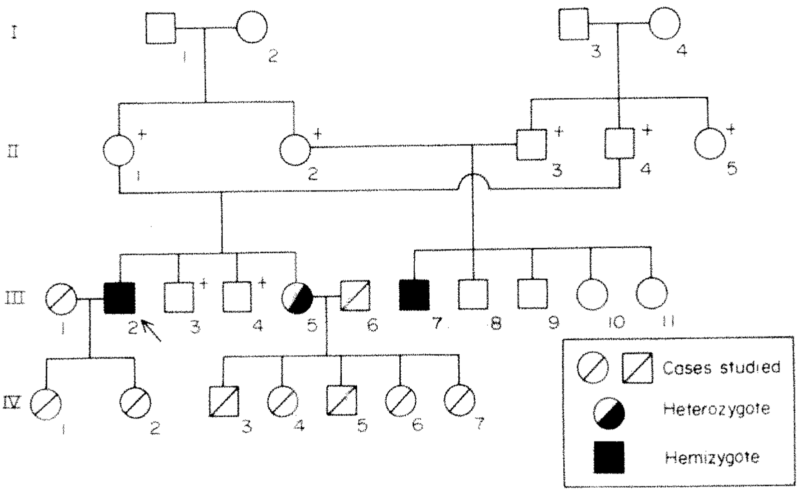

FIGURE 2. Pedigree of family L. +, Dead members of the family.

In our experiments, the results represent total activity, for which Triton X-100 was added at 0·1% to the incubation mixture of all the enzymes. It was established that under these conditions the activity, as measured, was proportional to the concentration of the enzyme and to the time of incubation.

Chemical studies

Neutral glycolipids were measured in the urinary sediment by the method described by Desnick, Sweeley & Krivit (1970).

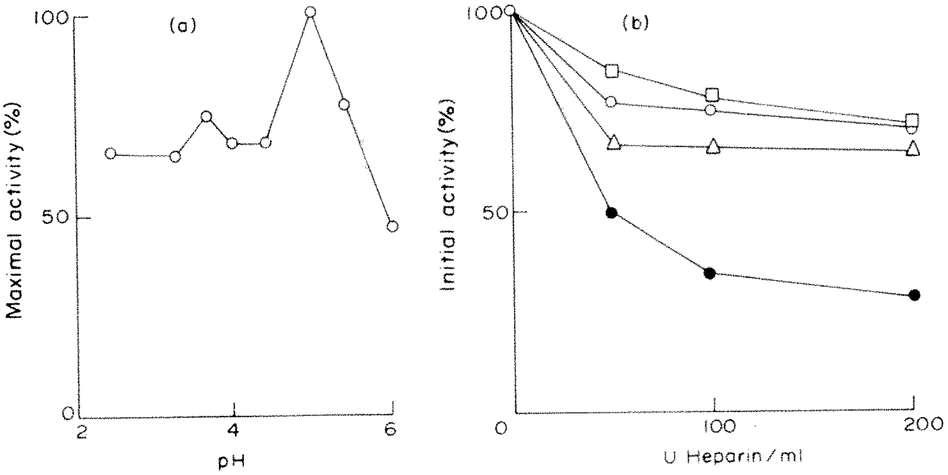

FIGURE 3. (a) pH activity curve of α-galactosidase in human polymorphs determined as described, except that the sodium acetate buffers were adjusted to the indicated pH. (b) Inhibition by heparin of human-leukocyte glycosidase activities. Human polymorph homogenates were prepared in 0·34 M sucrose in the presence of the heparin concentrations shown. Results are expressed as percentages of the activity obtained in the absence of heparin (controls). ●–●, β-glucoroidase; △–△, α-galactoxidase; ○–○, N-acetyl-β-glucosamidase; □–□, β-galactosidase.

Protein analyses were made according to the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

RESULTS

Properties of human-leukocyte α-galactosidase

All enzymatic studies were conducted using a 0·34 M sucrose solution in place of the 0·34 M sucrose–100 U heparin/ml previously used (Avila & Convit, 1970). The former was preferred because with 100 U heparin/ml in the homogenizing medium a strong irreversible inhibition of glycosidase activities had been found (Fig. 3b).

The pH activity curve (Fig. 3a) shows maximum activity at pH 5·0; a sharp drop in enzyme activity was observed at pH values above 5·0.

The two patients hemizygous for Fabry's disease showed a discrete residual α-galactosidase activity in peripheral PMN leukocytes. These results are similar to those of Romeo & Migeon (1970, 1971) and Brady, Uhlendorf & Jacobson (1971), who have reported a residual α-galactosidase activity between 11 and 20% of control values in fibroblasts from hemizygous subjects.

Furthermore, Romeo & Migeon (1971) indicated that the residual activity in Fabry cells represented another non-specific α-galactosidase enzyme, based on heat inactivation studies.

Clinically, these patients have typical skin lesions with minimal renal impairment (discrete proteinuria with normal endogenous urea and creatinine clearance), although they show greatly increased values of trihexosylceramides in urinary sediment. Both our hemizygous patients are very similar to patient 3 (generation IV) described by von Gemmingen *et al.* (1965); this patient presented similar clinical features, with normal urea and creatinine clearance, at the age of 41.

TABLE 2. Enzymatic activities in leukocytes from normal subjects and from patients with Fabry's disease

Position in pedigree	Age (years)	Nanomoles substrate hydrolyzed/mg protein/h			
		α-galactosidase	β-acetylglucosaminidase	β-glucuronidase	β-galactosidase
Controls (20) (Range)	3–60	32 (22–40)	425 (295–502)	225 (169–270)	44 (30–62)
			Hemizygous for Fabry's disease		
III, 2	45	7	296	217	43
III, 7	58	7	707	277	39
			Heterozygous for Fabry's disease		
III, 5	50	18	371	225	38
			Obligate heterozygotes for Fabry's disease		
IV, 2	7	35	283	226	32
IV, 1	4	26	301	228	35
			Relatives of patients with Fabry's disease		
IV, 3	10	19	199	154	31
IV, 4	13	33	352	226	36
IV, 5	14	28	220	146	51
IV, 6	15	36	378	222	46
IV, 7	16	25	305	173	35

On the other hand, one female patient heterozygous for Fabry's disease showed values of α-galactosidase activity between those of the hemizygous males and those of the control subjects (Table 2); this value is compatible with the percentages reported by Kint (1970) in patients heterozygous for Fabry's gene. This patient also has moderately increased values of trihexosyl ceramide and digalactosyl ceramide in the urinary sediment (Table 3) and bilateral corneal opacities on slitlamp examination.

The obligate heterozygous patients, daughters of the index case described above, differ in several respects from classic patients heterozygous for Fabry's gene. The most conspicuous departure from the usual biochemical picture is the normal value of α-galactosidase activity in peripheral PMN leukocytes, and normal urinary excretion of trihexosyl and digalactosyl ceramide. Clinically these two girls do not present any of the features reported in patients heterozygous for Fabry's disease.

TABLE 3. Concentrations of glycosyl ceramides in urinary sediment from normal subjects and of patients with Fabry's disease

Position in pedigree	Nanomoles of glycosyl ceramides/24 h					
	Galactosyl ceramide	Glucosyl ceramide	Digalactosyl ceramide	Lactosyl ceramide	Trihexosyl ceramide	Tetrahexosyl ceramide
Controls (13)	<1	8–15	<1	10–23	9–32	12–19
III, 2	14	32	36	19	416	59
III, 7	12	46	63	63	682	37
III, 5	4	15	16	11	151	24
IV, 2	<1	10	<1	22	19	12
IV, 1	<1	11	2	16	10	12

TABLE 4. Blood group serology of family L

Position in pedigree	Kinship	Blood groups												
		ABO	D	C	E	c	e	M	N	Fy^{b}	Le^{a}	s	K	k
III, 2	Index case	O	+	+	−	+	+	+	+	+	−	+	−	+
III, 1	Wife	A	+	−	+	+	+	+	+	−	−	+	−	+
IV, 1	Daughter (J.L.)	A	+	−	+	+	+	+	+	−	−	+	−	+
IV, 2	Daughter (J.L.)	O	+	−	+	+	+	+	+	−	−	+	−	+

The normal clinical and biochemical findings in these two obligate heterozygotes suggested several possible explanations for the differences between classical heterozygotes and these two daughters of our index case:

(a) Illegitimacy. This possibility was studied by blood group serology; the findings are compatible with the index case being the father of the two girls (Table 4). Furthermore, there are some phenotypic characteristics common to the two girls and the index case.

(b) That the girls are genetically XO, having lost the mutant X chromosome. This was excluded by sex chromatin studies.

(c) That the girls are indeed heterozygotes, but are still too young for overt clinical symptoms and biochemical manifestations. Against this possibility are the findings of de Groot (1970), who found symptoms of Fabry's disease in three typical heterozygous children as young as 5 and 8 years. Moreover, Desnick *et al.* (1971) have recently shown that the evaluation of glycosphingolipid concentrations in urinary sediment allows the detection of changes in renal tissue composition before clinical manifestations appear, as demonstrated by the early detection of a 2-day-old hemizygote (Philippart, Sarlieve & Manacorda, 1969), and asymptomatic heterozygotes for Fabry's disease (Philippart *et al.*, 1969; Desnick & Krivit, 1968). Philippart *et al.* (1969) have also reported that the urinary ceramide trihexoside excretion does not increase with age. These results as a whole suggest that even if these two obligate heterozygous girls did not yet have the typical clinical lesions characteristic of heterozygotes for Fabry's disease, they must excrete increased amounts of ceramide di- and trihexoside and should have intermediate levels of α-galactosidase activity in PMN leukocytes.

(d) Unusual Lyonization with a majority of the relevant anlage cells having the normal X chromosome as the active one. This hypothesis seems to us to be the most plausible explanation for the normal α-galactosidase levels in the two obligate heterozygous daughters.

Finally, Sweeley *et al.* (1971) have recently discussed briefly the problems which cannot be rationalized with the apparent genetics in Fabry's disease; these included the accumulation of a second glycosphingolipid (gal-gal-cer) which is not hydrolysed by the same ceramide trihexosidase, the complete absence of one of the enzymatic activities (pH 7·2) in plasma of heterozygotes, and the accumulation of acid mucopol-saccharides in cultured skin fibroblasts from Fabry's hemizygotes.

We believe that this paper further establishes the necessity for a clearer understanding of the apparent genetics of Fabry's disease.

ACKNOWLEDGMENTS

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A previously undescribed hereditary hair anomaly (pseudo-monilethrix)

B.BENTLEY-PHILLIPS AND MARGARET A.H.BAYLES

Department of Dermatology, University of Natal,
and King Edward VIII Hospital, Durban, South Africa

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SUMMARY

A new term 'pseudo-monilethrix' is suggested for an unusual form of 'beaded' hair, found in many members of a large Caucasoid family. The abnormality bears a resemblance to classical monilethrix but is not the same condition.

Other associated anomalies are formes frustes of pili torti and trichorrhexis nodosa. These disorders are traced through four generations and appear to have been inherited as an autosomal dominant trait. The clinical features and prognosis are discussed and the results of the examination of hairs under the light and scanning electron-microscopes are recorded.

Of the numerous abnormalities of hair structure described in the textbooks the three which we have encountered most frequently have been monilethrix, pili torti and trichorrhexis nodosa. The appearance of the hairs in the family studied in this investigation was quite unfamiliar, and perusal of the literature revealed no record of similar abnormalities. We have been unable to classify these anomalies, but as the main one bears a superficial resemblance to classical monilethrix we have tentatively termed the condition 'pseudo-monilethrix'. Figs. 1 and 2 illustrate the obvious differences.

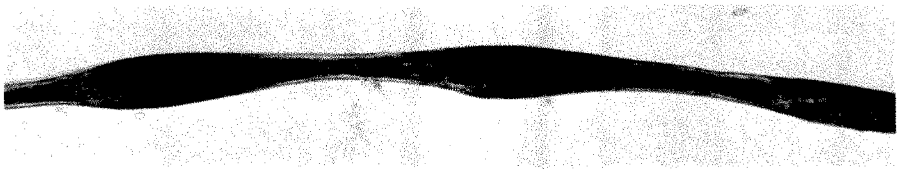

FIGURE 1. Classical monilethrix.

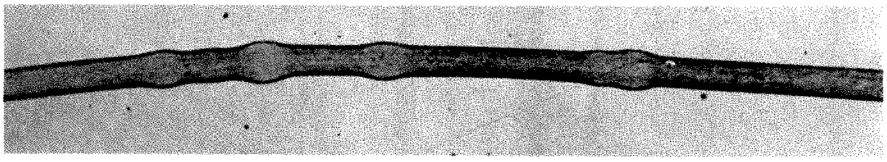

FIGURE 2. 'Pseudo-monilethrix' hair.

Reprint requests to: Dr B.Bentley-Phillips, 708 Eagle Building, West Street, Durban, Natal, South Africa.

CASE REPORTS

Our first 3 examples of pseudo-monilethrix were:

Case 1

Sandra H., a 12-year-old white girl with a 2 year history of progressive thinning of scalp hair, culminating in a large, almost bald area over the vertex and occipital regions. Clinically, the hair was sparse and thin, but there was no obvious evidence of any skin, scalp or hair disease, and only on microscopic examination of the hairs was structural abnormality detected (III: 46 in pedigree).

Case 2

Mrs P.H. (Sandra's mother). A healthy, 34-year-old woman who was surprised when similar structural abnormalities of her hair were demonstrated under the microscope. She considered her hair to be unruly and difficult to manage, but she had been unaware of any hair disorder, and no baldness had ever been present (II: 16 in pedigree).

Case 3

Mrs S.G. (Sandra's grandmother). A healthy woman aged 68 who had never noticed any abnormality of her hair, but who stated that it had always been rather dry and grew at an excessive rate, necessitating frequent trimming. 'Beading' of hairs, similar to that of her daughter and granddaughter was demonstrated on microscopy (I:6 in pedigree).

INVESTIGATIONS

After the same anomaly had been discovered in hairs obtained from Sandra's sister and two brothers, a full investigation of this family was undertaken, and with the invaluable help of Mrs H. a pedigree was drawn up.

The original immigrants, Charles and Sarah J. (now deceased), had been born in London and emigrated to South Africa in 1886. The majority of their descendants had settled in Natal and a total of eighty-two members in four generations was traced, consisting of seven children, nineteen grandchildren, fifty-three great-grandchildren and two great-great-grandchildren (Fig. 3).

All available members of this large family were examined, but those whom we were unable to see personally were sent detailed questionnaires and asked to record any defects of hair, skin, nails, teeth or eyes, the colour and texture of hair and also the presence of any congenital abnormalities (anatomical, mental, etc.). Generous samples of scalp hair were requested to be returned with the completed forms, and a total of seventy-six records and hairs was obtained. Details were analysed and hair samples examined under light and scanning electron microscopy.

RESULTS

Analysis of replies to questionnaire

The majority of the affected subjects were unaware of any major hair abnormality. Direct questioning produced a few vague comments, e.g. 'dry hair', 'greasy hair', 'fine hair', 'difficult hair'. Enquiry into associated epidermal defects was also of little value as complaints were mainly subjective, e.g. dry skin, brittle nails or poor teeth, and no regular pattern of physical or mental abnormalities emerged.

(a) No subjects were born with lanugo hairs.

(b) Eyebrows and eyelashes were unaffected.

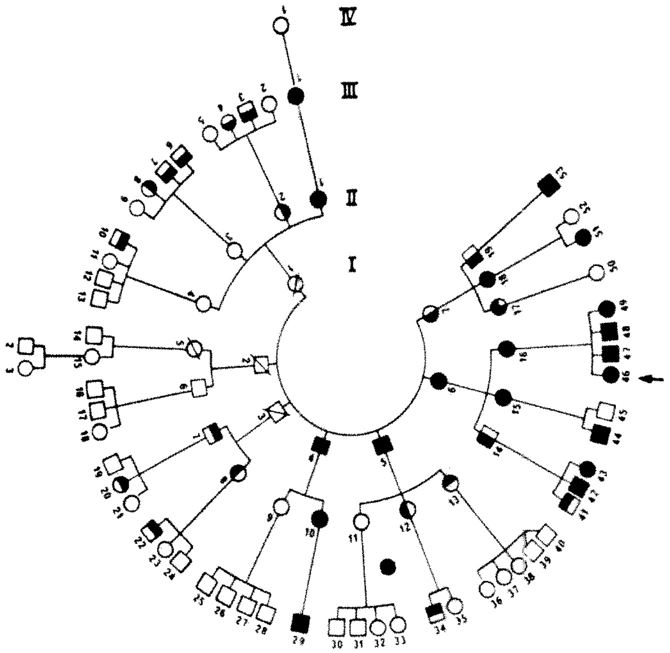

FIGURE 3. Family pedigree. ○, Female; □, male; ■, pseudo-monilethrix; ⬒, trichorrhexis nodosa; ◧, pili torti. ⧄, ⊘, No information available.

(c) Body hair was essentially normal in both males and females.

(d) In cases in which the baldness and sparseness of hair were noticed, the age of onset was in childhood, from approximately 8 years to puberty.

(e) There were no cases of premature baldness in adult males.

(f) There had been excessive brushing of the hair, as many of the older members had been trained from childhood to brush 200 strokes daily.

(g) Adult females now have their hair washed and set professionally once or twice a fortnight, the hair being back-combed. This procedure is said to be unavoidable when attempting to groom 'difficult' hair.

(h) Growth rate is reputed to be normal, but the proband's grandmother (I: 6) has to have her hair cut very frequently as it grows so rapidly. All other persons reported 'normal' rate of growth.

(i) The hair colour in this family is predominantly fair (Table I).

(j) No cases of keratosis pilaris were found (a condition commonly associated with classical monilethrix).

(k) Where hair is sparse and thin there is a tendency for individuals to try to 'stimulate' growth by

TABLE I. Hair colour in family members

	Fair	Medium	Dark	Total
Beaded hair	16	3	0	19
Pili torti	3	5	0	8
Trichorrhexis nodosa	9	2	0	11
Normal	27	8	3	38

TABLE 2. Frequency of three types of anomalous hair in the family

Numbers examined		Beaded hair	Pili torti	Trichorrhexis nodosa	Unaffected
Males	36	9	2	9	17
Females	40	10	6	4	21
Total	76	19 (24·1%)	8 (10·5%)	13* (17%)	38

* Double pathology in two cases: II, 17 (pili torti and trichorrhexis nodosa); III, 42 (beaded hair and trichorrhexis nodosa).

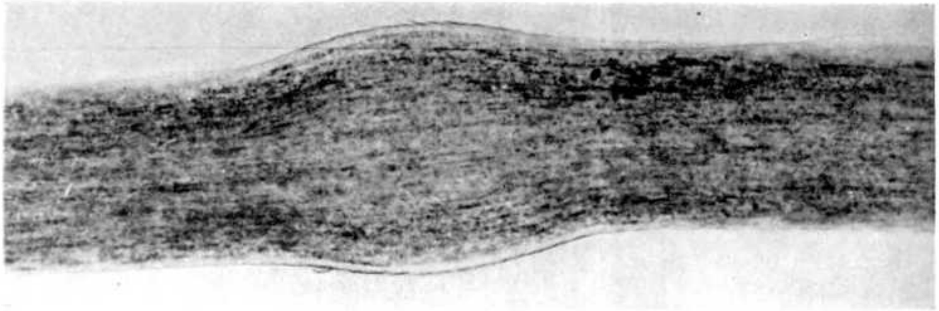

FIGURE 4. 'Pseudo-monilethrix' node.

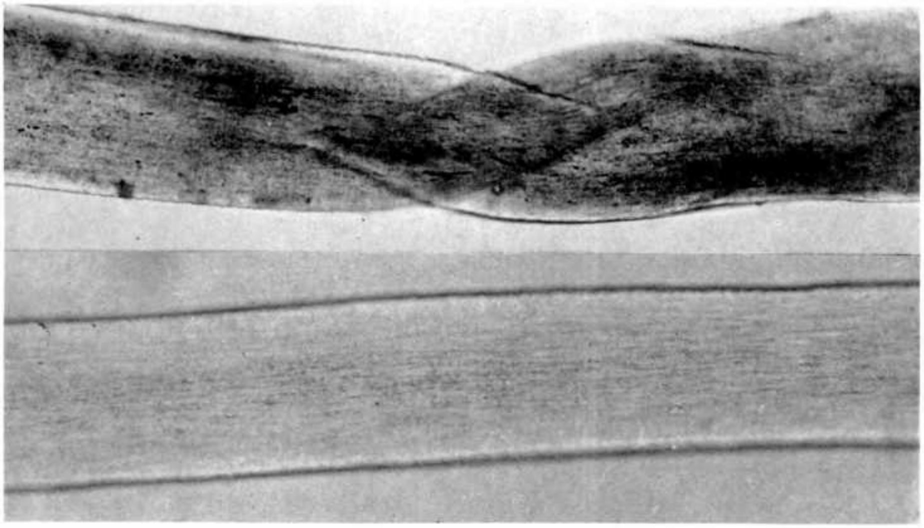

FIGURE 5. Forme fruste of pili torti and a normal hair.

massage and vigorous brushing. This leads to breakage of hairs, further loss and re-doubled efforts at 'stimulation'.

Examination of hairs

Of the eighty-two members of the family three were deceased and three unco-operative. Hair from the remaining seventy-six was examined and the main anomalies are detailed in Table 2. In summary, nineteen (24·1%) showed the curious 'beading' of hair (Fig. 4), eight (10·5%) showed twisted hairs of an unfamiliar type (Fig. 5), thirteen (17%) showed an unusual form of broken hairs (Fig. 6). Double pathology was observed in two cases. We observed other defects from time to time, kinks, bands, breaks and ridges, but were particularly intrigued by single or double 'indentations' which occurred frequently and which were completely unfamiliar to us (Fig. 7).

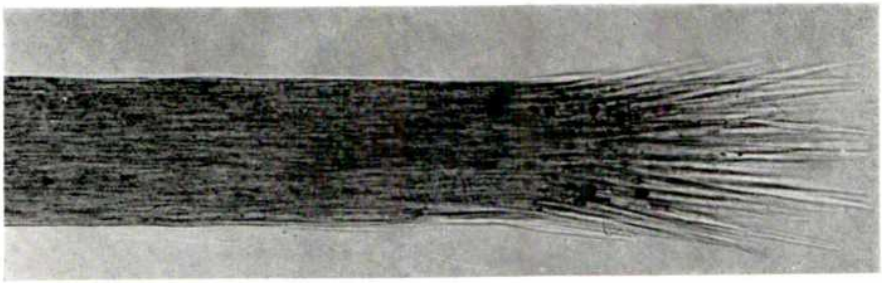

FIGURE 6. Unusual type of trichorrhexis nodosa.

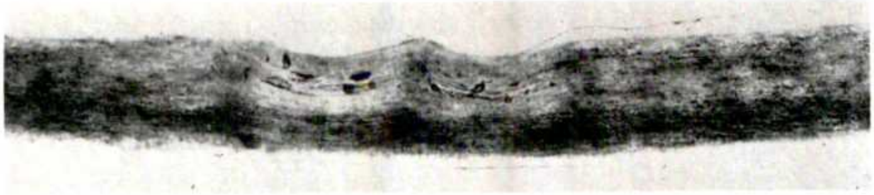

FIGURE 7. 'Indentations' of abnormal hair.

'Beaded' hairs. Under light microscopy nodal swellings (Figs. 2 and 4), measuring 0·75–1 mm in length were seen, interrupting the hair shafts at irregular intervals, the internodes being of normal thickness. The hairs bore a superficial resemblance to classical monilethrix but were obviously not the same condition.

Under scanning electron microscopy, using magnifications from 150–3000 it became obvious that the 'nodes' seen with light microscopy had been optical illusions, and that they were actually indentations in the hairs, the sides of the depressions protruding beyond the normal diameter of the shafts. Scale pattern of nodes and internodes was essentially normal (Fig. 8).

Twisted hairs. These hairs were twisted irregularly, the rotation varying from 25–200°, with no flattening of the shafts, whereas in classical pili torti hairs show regular twists of 180° with flattening (Fig. 5).

Broken hairs. In classical trichorrhexis nodosa the breaks and 'brushes' occur at points of cuticular

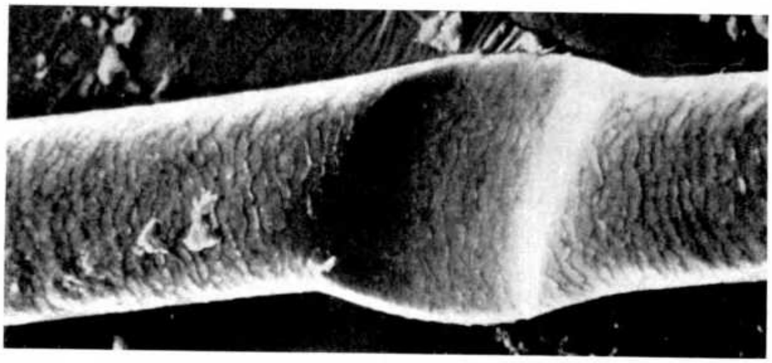

FIGURE 8. SEM appearance of 'depressions' (× 250).

fissuring and subsequent cortical damage, whereas the breaks in these abnormal hairs appear to be on normal shafts (Fig. 6).

When magnified with the scanning electron microscope, the twisted hairs and broken hairs did not show any unexpected features. The structure and scale pattern were normal.

DISCUSSION

Monilethrix (beaded hair), first described by Walter Smith of Dublin in 1879, is a genetically determined defect of hair structure characterized by repeated nodes and internodes along the length of the hair shaft. The condition is inherited as an autosomal dominant trait. Hairs are described as having fusiform spindle-shaped or elliptical swellings, 0·7–1 mm apart, separated by narrow portions. The nodes are considered to be the normal diameter of the shaft, the internodes representing atrophic parts. The condition is rare but has been well documented and investigated (Tomkinson, 1932; Cockayne, 1933; Baker, 1962; Heydt, 1963, 1964; Solomon & Green, 1963; Malt, 1965; Rodermund, 1969).

Pili torti, also a genetically determined hair anomaly, is characterized by twists of 180° and flattening of the hair shafts, well described by Hellier (1940). Recently a pedigree of seven generations has been investigated by Walker (1970).

Trichorrhexis nodosa is considered to be a phenomenon caused by mechanical trauma (Ebling & Rook, 1968) and is not determined genetically. It is characterized by fractures of the hairs at weak points resulting in the typical appearances of 'brush' ends.

Nomenclature

The three major hair anomalies encountered in the family under consideration were different from these classical hair disorders. We did not consider that the twisted or broken hairs, although unusual, required new titles, but as the hairs with the illusory 'nodes' were so unique, a new designation was deemed necessary. The condition was obviously not classical monilethrix, and the term 'beaded hair' could not be adopted as this is already a synonym for this anomaly. However, we felt that any new term should indicate a resemblance to monilethrix, and having considered titles such as 'monilethroid' and 'monilethrixoid' we decided finally upon 'pseudo-monilethrix'.

Essential differences between monilethrix and pseudo-monilethrix are detailed in Table 3.

TABLE 3. Comparison of classical monilethrix and pseudo-monilethrix

Feature	Classical monilethrix	Pseudo-monilethrix
Regularity of 'nodes'	Striking regularity	Irregular spacing
Diameter of 'nodes'	As normal shaft	Greater than normal shaft
Diameter of internode	Less than normal shaft	As normal shaft
Light microscope	Fusiform swellings	Optical illusion of nodes
SEM nodes	Normal transverse scales	Normal transverse scales
SEM internodes	Longitudinal scales and ridging	Normal transverse scales
Pili torti	Associated at times	Associated—formes fruste
Trichorrhexis nodosa	Associated frequently	Associated—unusual breaks
Colour of hair	Predominantly dark?	This family predominantly fair
Age of onset	Infancy/early childhood	8–14 years in this study
Keratosis pilaris	Common	Not found in this series
Sex	M > F	M = F
Inheritance	Autosomal dominant	Autosomal dominant
Clinical	Weak sparse thin hair	Varies from sparse to normal

Genetic aspects

From a study of the pedigree it is evident that there is no sex limitation in the incidence of pseudo-monilethrix, and the pattern of inheritance supports autosomal dominant transmission with variable penetrance. There were nineteen individuals with the 'nodes' and although this is only half the number expected of dominant transmission, when the three anomalies of hair structure are considered in the present family the ratio of normal to abnormal is exactly 1 : 1. Our conclusions have been verified by a geneticist.

This pattern of inheritance appears to be in line with the mode of transmission of other inherited hair abnormalities, e.g. monilethrix and pili torti, but it is generally believed that trichorrhexis nodosa is a phenomenon caused by mechanical trauma. The indication from the family study is that an underlying genetic defect may predispose some individuals to excessive fragility of the actual hair shafts, and not only at weak points or constrictions.

During the past year we have taken the opportunity of examining the hairs of large numbers of apparently normal persons in addition to those complaining of 'poor hair', 'brittle hair', 'thin hair' or diffuse types of alopecia, and have come to the conclusion that the condition we have termed pseudo-monilethrix is not uncommon and may be recognized readily if looked for. We now have several small pedigrees with this condition, and a number of cases where hair samples of relatives have been unobtainable. The anomaly has been seen amongst British, Germans, Dutch, South Africans and Indians, but not so far in Africans. An examination of the hairs of 500 unselected Indians and those of 1000 unselected Europeans produced nineteen examples of pseudo-monilethrix and 11 of trichorrhexis nodosa. No cases of pili torti were seen.

It is obvious that clinical features may be minimal in this condition, many sufferers (and dermatologists) being oblivious of the hair abnormality. The patients consider themselves unfortunate in having 'poor quality' hair and waste time and effort in attempting to 'stimulate' the growth by vigorous brushing and massaging. Money is also wasted on hair restorers, conditioners, and expensive trichological procedures, some of which are more traumatic than others but all equally valueless.

We are of the opinion that pseudo-monilethrix may be the aetiological factor underlying many cases of diffuse alopecia, and have observed that once the patient is diagnosed, reassured, shown the hairs under the microscope and understands that the 'nodes' represent weak spots in the hair shafts, the prognosis of this type of alopecia becomes favourable. Improvement, regrowth of hair and complete disappearance of bald areas may be anticipated once 'stimulation' ceases. Sandra H.'s hair is now apparently normal, and as can be seen from the 'before and after' photographs of an Indian child, V.C. aged 6 (Figs. 9 and 10), regrowth has been complete. Treatment consists of explanation, reassurance, and warnings against all types of 'stimulating' procedures.

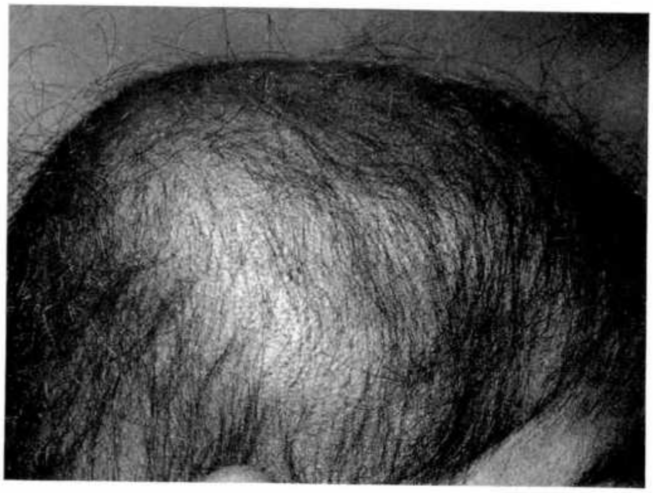

FIGURE 9. Clinical state of Indian child before 'treatment'.

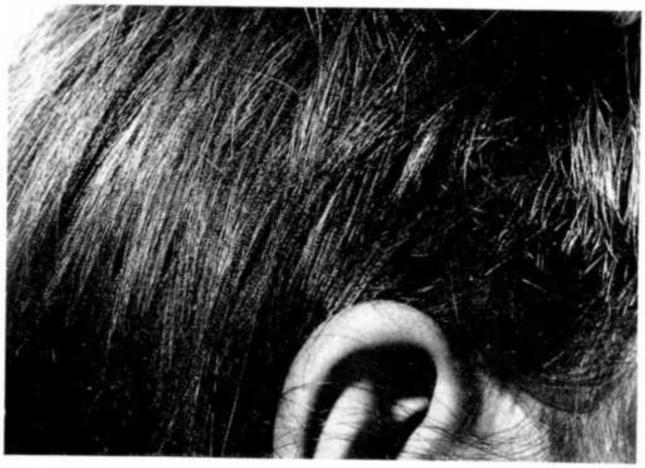

FIGURE 10. Clinical state of child after 'treatment'.

We considered the results of this investigation worth reporting as by recognizing the condition many individuals can be helped physically, psychologically and financially.

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Skin complications in immunosuppressed patients: follow-up of kidney recipients

S.HAIM, R.FRIEDMAN-BIRNBAUM, O.S.BETTER AND S.TUMA

Departments of Dermatology and Nephrology, Rambam Government Hospital and Aba Khoushi School of Medicine, Haifa, Israel

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SUMMARY

Thirty-five patients who received thirty-seven kidney transplants (two patients received live; the rest cadaver kidneys) were followed up for skin complications.

There were thirty-nine instances of overt skin infection. In twenty-one instances we encountered fungal, in ten pyogenic and in eight viral infection. The course of these infections was more severe and more protracted than is usual in other patients. In nine instances, the skin infection contributed directly to the death of the patient. One patient developed Kaposi's sarcoma and another pyoderma gangrenosum.

This study suggests that immunosuppressive treatment, which all our patients were continuously receiving, enhances their susceptibility to infection and to malignancy and may have been an aetiological factor in pyoderma gangrenosum.

Patients who have undergone organ transplantation are maintained for a long period on immunosuppressive therapy. This, in turn, may damage the host defence mechanisms and alter the course of various pathological processes.

During the period December 1965 to March 1972, thirty-seven kidney transplantations on thirty-five patients were performed in our hospital. The purpose of this paper is to report the dermatological complications encountered in these patients.

RECORDS OF CASES

Thirty-five kidney recipients were followed up, nine females and twenty-six males. Their average age was 29 years. The youngest was a boy aged 7 and the eldest a man aged 55. Of the thirty-five, seventeen died; in six the transplanted kidney failed and the patients were returned to haemodialysis. The follow-up period of the latter cases is from 7 months to over 4 years. All the grafts except two were cadaveric kidneys.

Immunosuppressive regimen

Hydrocortisone 500 mg and Endoxan 200 mg in glucose solution were infused intravenously immediately before operation. After operation, hydrocortisone 500 mg daily was administered for the first week.

Later, the dose was gradually reduced and oral prednisone reaching 30 mg daily by the end of 3 months was substituted for the intravenous hydrocortisone. A further reduction of this dose was later considered. In addition, Imuran (azathioprine) 50 mg was given daily during the anuric period and the dose was raised to 200 mg when the transplant started to function. During the first post-operative week the recipient also received antibiotics.

In case of rejection, the daily dose of hydrocortisone was temporarily raised to 1500–4000 mg. In addition, 150 rad of X-radiation were given daily to the transplant for 4 consecutive days.

Dermatological complications

The dermatological manifestations presented by our patients were as follows: infection in thirty-nine instances, Kaposi's sarcoma in one and pyoderma gangrenosum in one. Thus, the most frequent complication was infection. The course of these infections, whatever the aetiology, tended to be more severe, protracted and more resistant to treatment than is usual in other patients and infection was the immediate cause of death in nine of the seventeen fatal cases. Details of the clinical types of skin infections are shown in Table 1.

TABLE 1. Clinical presentation of skin infections

Type of infection	Frequency
Pyogenic	
Cellulitis	7
Erysipelas	2
Furunculosis	1
Mycotic	
Candidiasis	16
Dermatophytosis	4
Mucormycosis	1
Viral	
Herpes simplex	4
Verruca vulgaris	2
Generalized herpetic rash	2

The most frequent type of infection was candidiasis. It usually presented in its intertriginous forms and as stomatitis, glossitis, perleche, onychia and paronychia. In two instances it occurred in the form of vaginitis and in one as scattered, grouped, vesicular and exudative patches.

On seven occasions we encountered a frank Gram-negative septicaemia, on four systemic fungal infection (one mucormycosis and three candidal) and on two a systemic viral infection. The clinical manifestations of these systemic infections were often masked and a high index of suspicion was, therefore, necessary in detecting them. This was well demonstrated by a patient with rhinocerebral mucormycosis. The case has already been discussed elsewhere (Haim *et al.*, 1970). The recipient was a woman aged 23. From the second post-operative day, she complained of pain over the maxillae. Repeated extensive investigations failed to reveal any abnormalities. Five weeks after transplantation, a rapidly progressive oedema developed around the bridge of the nose. This was followed by paralysis of the right 3rd, 5th, and 7th cranial nerves. The skin of the face became swollen. A necrotic inflammation of both nostrils appeared and rapidly involved the zygomatic areas, and the nasal cavity was filled with purulent fluid. Later, bilateral exophthalmos developed, followed by loss of vision. Repeated cultures of the nasal discharge showed a growth of *Klebsiella* but failed to reveal a suspected fungal infection. The patient's condition deteriorated rapidly and she died 6 weeks after operation. The kidney graft continued to function normally until death.

Autopsy examination revealed thrombosis of the cavernous, petrosal, maxillary and the sphenoid sinuses on the right side. Both internal carotid arteries were occluded with white thrombus. The thrombus, as well as the affected sinuses and the affected portion of the skin, were infiltrated with hyphae typical of mucormycosis. The hyphae were pleomorphic, large, non-septate and showed numerous lateral branches.

Besides infection we encountered two other dermatological disorders which may be related to immunosuppression. The first was Kaposi's sarcoma and the second protracted penile ulceration compatible with pyoderma gangrenosum. The following is a brief description of these cases.

Case 1. H.C. was a man aged 24. He suffered from irreversible kidney failure due to chronic glomerulonephritis, and in March 1968 a cadaveric kidney was transplanted. At present, 4½ years after the operation, the graft functions well with a creatinine clearance of 60 ml/min.

A year ago, and while he was receiving immunosuppressive therapy, a group of red-purple nodules and indurated plaques appeared in the skin of his left lower leg which became markedly oedematous. Histologically, the lesions showed structural changes consistent with those of Kaposi's sarcoma. The daily dose of azathioprine was reduced from 100 to 50 mg and following treatment with 1000 rads of X-radiation the lesions regressed.

Since then, no sign of visceral involvement has been detected. However, crops of new lesions continue to occur in the skin on different parts of his body.

Case 2. The recipient was a man, aged 42 years. During the tenth post-operative month, a painful reddish swelling appeared on the glans penis. The swelling rapidly broke down and an intractable, painful, burrowing ulcer developed. The ulcer steadily enlarged and reached a diameter of 1·5 cm. It became surrounded by violaceous and erythematous zones and had irregular, rounded and necrotic edges and a base covered by purulent slough. No enlargement of regional lymph nodes was detected.

The laboratory investigations, including dark-field, bacteriological studies and serological tests for syphilis, were non-contributory. No pertinent abnormality was detected in the physical examination of the other systems of the body. Biopsy from the base of the ulcer showed absence of the epidermis. The upper dermis was necrotic and permeated by an acute inflammatory infiltrate. Deeper in the dermis, the infiltrate was more chronic in nature with granuloma formation. There was an increase in the number of the blood vessels, and in some of them proliferation of the endothelium was noted.

Systemic and topical antibiotics failed to bring an improvement and the ulcer lasted more than 3 months before it healed with scarring. Meanwhile, two new similar lesions developed in the groin. For a year new lesions continued to appear on the penis and in the groin while the old ones healed with scars. At the end of this period, and following final rejection of the transplant, the patient was returned to haemodialysis and the immunosuppressive drugs were discontinued. The existing ulcers healed gradually and no further lesions were noted during the next year, which was ended by the patient's death following a cerebrovascular accident.

DISCUSSION

Immunosuppressive therapy carries with it many dangers to the patients. The most common have been infections (Hill *et al.*, 1967; Park, Goltz & Carey, 1967; Rifkind *et al.*, Rifkind, Faris & Hill, 1966; Rifkind, 1965), and of these, the most serious are those caused by opportunistic fungi (Hood *et* *al.*, 1965; Reynolds, Walls & Pfeiffer, 1966). Hill *et al.* (1967) analysed the cause of death in sixty patients who received kidney, liver and spleen grafts. Sepsis was responsible for four of sixteen deaths which occurred during the first 24 post-operative days, whereas death was clearly related to infection in thirty-eight of the forty-two patients who died 25 days or more after surgery. These late infections were mostly due to opportunistic, mainly fungal, infection rather than to common pathogens. In our group of patients, only one of the seventeen deaths was directly related to fungal infection.

Another point of concern in immunosuppressed patients is malignancy. The immune cell-mediated response is widely believed to be important as a defence mechanism in preventing the growth of spontaneously occurring neoplastic mutations (Burnet, 1965). Smith (1968) refers to a high incidence of spontaneous growth, especially of the lymphoreticular system, in patients with acquired or congenital immunological disorders. The same disorders are claimed by some to be the cause of the rising frequency of malignancies with advancing age (Cammarata, Rednau & Fennel, 1967). Experimental animals treated with antilymphocytic serum or which underwent thymectomy show a high incidence of chemical or virus induced neoplasia (Low, 1969). Beside their interference with the immune defence of the body, azathioprine and related compounds are known to be mutagens in man (Jensen, 1967). Their effect on somatic cells might produce structural chromosomal changes which could result in mutant neoplastic lines (Leb & Howell, 1971) or render the cells susceptible to transformation by other oncogenic agents, such as virus (Todaro & Green, 1964). It is reasonable, therefore, to assume that spontaneous neoplasms may be more frequent in the group of patients requiring long-term immunosuppression. Recent studies report several instances of malignancy in association with immunosuppressive therapy in patients who have received renal transplants (Wilson *et al.*, 1968; McKhan, 1969; Simmons, Kelly & Tallent, 1970; Kin & Williams, 1972), including a patient with Kaposi's sarcoma (Seigal *et al.*, 1969) and it can be speculated that our patient provides a further example.

The third noteworthy finding in our data is the pyoderma gangrenosum in Case 2. The aetiology of this disorder is still undecided. In most cases, it has been related to visceral lesions, especially ulcerative colitis (Haim, Freidman-Birnbaum & Alroy, 1971). Jablonska (1964) assumes that the disease could be the result of an hyperergic reaction against bacterial lipopolysaccharides. Long & Uesu (1964) observed in two patients with pyoderma gangrenosum rejection of an autoskin transplant and a delayed hypersensitivity to intradermal injection of the patients own leucocytes. The ulcers in our case occurred while the patient was receiving immunosuppressive therapy and they apparently cleared after discontinuation of the drugs. We may speculate, therefore, that the disease in this case was connected in some way with immunosuppression.

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Herpes zoster complicating myocardial infarction

M.E.BENAIM* AND D.R.SMITH

Department of Cardiology, The General Infirmary, Leeds

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SUMMARY

A patient is described who developed a zoster eruption over the left sixth cervical dermatome whilst convalescing from a myocardial infarction. The association is reviewed and the diagnostic implications are stated.

Involvement of the upper limbs by the eruption of herpes zoster is uncommon, and affection of the sixth cervical dermatome is particularly rare (Head & Campbell, 1900; Hope-Simpson, 1965). Its appearance in a patient soon after a myocardial infarction presented aetiological and diagnostic problems of interest.

CASE REPORT

A woman aged 70 was admitted to the Leeds General Infirmary in May 1971 following a severe attack of central chest pain radiating into both shoulders, particularly the left, and down the whole of the left arm to the fingers. The pain lasted for 12 h, and was followed by an unpleasant feeling of 'pins and needles' affecting the radial aspect of the left forearm and hand which persisted for 24 h. There had been no recent contact with a case of varicella or herpes zoster.

Examination revealed a temperature of 38°C, nodal rhythm at a rate of 60 beats/min, moderate cardiomegaly and signs of early left ventricular failure. A recent anteroseptal myocardial infarction was confirmed on the basis of the electrocardiographic appearances and the serial changes in the serum levels of the heart enzymes. The ESR was 9 mm in the first hour.

Convalescence was uneventful until the beginning of the third week. She then became pyrexial once more, and for about 10 days the temperature remained around 38°C. During this time, the patient experienced continuous pain over the left shoulder, down the outer aspect of the left arm and hand. The pain was described as being 'heavy and burning' in character and similar to that which had accompanied the myocardial infarction. Movements at the shoulder were a little restricted and there was hyperaesthesia over the outer aspect of the left arm, but no other abnormal neurological signs were present. Radiology showed no abnormality in the left shoulder and only mild cervical osteoarthritis.

The possibility of a further myocardial infarction was considered but serial ECG tracings and enzyme studies showed no evidence of this. She was investigated as a case of PUO, but the only abnor-

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* Present address: Department of Medicine, Royal Victoria Infirmary, Newcastle-upon-Tyne, NE1 4LP.

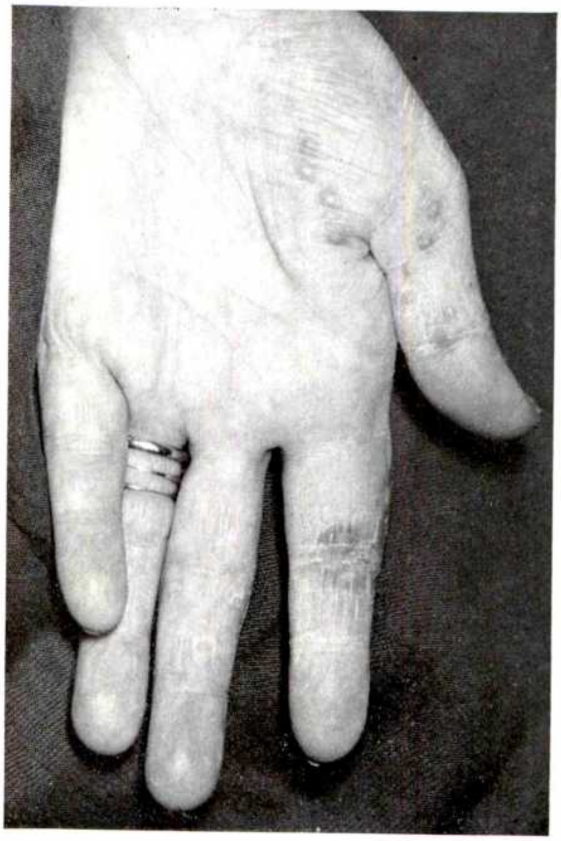

FIGURE 1. Herpes zoster affecting the left sixth cervical dermotome.

mality found was an ESR of 54 mm in the first hour. In due course the diagnosis became clear, for on the tenth day she developed a herpetic eruption affecting the left sixth cervical dermatome (Fig. 1). The appearance of the eruption coincided with remission of the pyrexia and a gradual return of the ESR to normal over the following week. Moderate post-herpetic neuralgia persisted for a further 6 weeks and gradually subsided.

DISCUSSION

Various authors have described the association of herpes zoster with ischaemic heart disease. In certain cases, the zoster eruption has preceded anginal pains over the same area by a period varying from a few months to a few years (Spillane & White, 1939). A second group of cases has been described in whom herpes zoster developed over areas previously affected by repeated attacks of anginal pains over a long period of time (Spillane & White, 1939; Horton, 1965). In the present case, the eruption followed a myocardial infarct.

The most likely hypothesis advanced to explain the pathogenesis of herpes zoster is that it is due to reactivation of latent varicella-zoster virus which has remained dormant in the body since the original attack of varicella (Hope-Simpson, 1965). The appearance of the zoster lesions in such close temporal and spatial relationship to the area affected by the referred cardiac pain makes it likely that irritation of the sixth posterior root ganglion by painful or other impulses from the diseased heart has been responsible for the reactivation of the dormant virus.

It seems probable that the development of a herpetic eruption over an area of the upper limb previously affected by referred myocardial pain may be yet another example of a 'trophic' manifestation complicating ischaemic heart disease. Among these may be mentioned the ecchymoses described by McKenzie (1893), unilateral Raynaud's phenomenon (Bard, 1921), trophic ulcers of the hands (Shapiro, Lipkis & Kahn 1947), the shoulder–hand syndrome extensively discussed and reviewed by Rosen & Graham (1963) and the demonstration of transient skin coolness within the distribution of anginal pain (Potanin, Hunt & Sheffield, 1970).

From the point of view of diagnosis, two points require emphasis. Firstly, it is important to record an electro-cardiogram when a zoster eruption over the chest or arms is encountered, as an underlying recent myocardial infarction may otherwise be missed. Secondly the reappearance of pain during convalescence from a recent myocardial infarction does not always signify continuing myocardial damage and other possibilities, both visceral and somatic, should be considered.

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Pharmacology and Treatment

Chronic muco-cutaneous candidiasis: new approaches to treatment

JANET M.HIGGS AND R.S.WELLS

Department of Dermatology, Guy's Hospital, London, S.E.1 and
Institute of Dermatology, St John's Hospital, Leicester Square, London WC2H 7BJ

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SUMMARY

Chronic muco-cutaneous candidiasis may be associated with several defined syndromes of immunodeficiency or may be the predominant feature in a group of diseases, some of which are genetically determined.

Current therapeutic approaches are discussed in relation to each clinical group, with particular reference to immunological reconstitution, correction of iron deficiency and other nutritional deficiencies. The effects of two new antifungal agents are considered, as well as the use of parenteral amphotericin B in a low dose. The possible use of combined therapy is outlined in patients who are more severely affected.

Chronic muco-cutaneous candidiasis may occur in association with a wide variety of disorders, as well as constituting the predominant clinical feature in a group of diseases. Therefore the need for careful investigation and accurate assessment cannot be over emphasized, because the approach to therapy may vary significantly in patients of different clinical groups.

At present some of the fundamental problems underlying chronic muco-cutaneous candidiasis are being recognized and more insight is being gained into the difficulties and the reasons for lack of success in treating these rare conditions.

It is also true to say that the best line of treatment has not been fully elucidated in many instances, because a number of alternative methods and new pharmaceutical preparations are still under evaluation. The purpose of this paper is to discuss some of these methods in the light of recently acquired knowledge and our own experience of patients in different clinico-genetic groups.

CLINICAL CLASSIFICATION

Patients with chronic muco-cutaneous candidiasis may be classified into two main groups; first, those in whom the infection is associated with one of the defined immuno-deficiency syndromes (Group 1), which are either congenital or genetically determined; secondly those who have chronic muco-cutaneous candidiasis as the predominant disorder (Group 2).

Group 1

Individuals in the first group usually fail to thrive and nearly always die early in childhood. The progress of the disease is dominated by severe and recurrent infections of various types. Chronic muco-cutaneous candidiasis may be a prominent associated feature of some of these conditions and is usually confined to those in whom thymic development is defective, resulting in impaired cell-mediated immunity. In such cases this may be systemic infection. The disorders classified in this group include a number of primary immuno-deficiencies, for example Swiss-type agammaglobulinaemia (Tobler & Cottier, 1958), hereditary thymic dysplasia (Nezelof *et al.*, 1964) and Di George's syndrome. In the last there is a congenital disorder resulting from a developmental defect of the third and fourth branchial pouches (Di George, 1965).

Group 2

Individuals in the second group may develop chronic muco-cutaneous candidiasis early in childhood, but they usually survive at least to early adult life, and show chronic oral, skin and nail infection as the predominant clinical features. Many patients in this group have been shown to have a genetically determined disorder (Wells *et al.*, 1972), but others are included who develop the disease late in life and in whom the condition is acquired.

Patients in this group may be further sub-divided into one of four sub-groups, all of which have one feature in common, namely chronic oral candidiasis. This may be controlled, but is not cleared by topical antifungal agents.

The first sub-group includes individuals with familial chronic muco-cutaneous candidiasis (FCMC) in which the predisposition to chronic oral and nail infection is apparent at an early age and is inherited as an autosomal recessive trait.

The patients with FCMC differ significantly from the individuals in the second sub-group, who develop extensive cutaneous candidiasis, including granulomas in some instances. We have called this condition diffuse chronic muco-cutaneous candidiasis (DCMC) and although these patients are often also susceptible to recurrent bacterial chest infections and skin sepsis, they usually survive childhood and cannot be shown to have a primary immuno-deficiency of the type previously defined in Group 1.

The third sub-group consists of patients with the candida-endocrinopathy syndrome, that is chronic oral and nail candidiasis generally associated with hypo-parathyroidism and hypo-adrenocorticism. This is a genetically determined disorder inherited as an autosomal recessive trait (Sutphin, Albright & McCune, 1943).

Finally, chronic oral candidiasis occurring later in life, often after the age of 50, is usually acquired. It may be idiopathic, associated with a denture stomatitis or occasionally with an occult malignant condition. Cutaneous candidiasis is uncommon in this group and when seen, it is usually part of the rare syndrome of cutaneous candidiasis, myopathy and thymoma (Montes *et al.*, 1968). This classification is discussed in detail elsewhere (Higgs & Wells, 1973a).

The importance of this classification is stressed, not only as an aid to correct diagnosis, but particularly because of its relevance to the use of different therapeutic methods with different conditions. For example, in Group 1, treatment is directed primarily towards correction of the underlying immuno-deficiency, and in the few cases where some measure of success has been achieved resolution of the fungal infection has been observed.

This has led to the use of similar techniques in the treatment of the more severely affected individuals in Group 2, that is the patients with diffuse chronic muco-cutaneous candidiasis, who have defects in cell-mediated immunity which are apparently of a less fundamental type. However, the broader clinical spectrum observed in Group 2 as a whole, gives considerably wider scope for variation in therapeutic methods whereby substantial clinical improvement may be achieved by relatively simple means.

TREATMENT OF THE DEFINED SYNDROMES OF IMMUNO-DEFICIENCY. GROUP 1

Chronic muco-cutaneous candidiasis occurring in association with these disorders may respond initially to topical antifungal agents such as nystatin and oral amphotericin B. However, it is usually recurrent and is subject to the underlying disease, the treatment of which is generally unsatisfactory. The only possible way of treating a primary immuno-deficiency involving the thymus dependent system, is by reconstitution with immunologically competent cells, but in most conditions of this type several factors may preclude the success of such procedures.

Grafts of bone marrow cells and foetal tissues, such as liver and thymus, have been used on several occasions and in some instances restoration of immunological responsiveness has been achieved to a marked degree (*Bulletin of the World Health Organization*, 1971; Kay, 1972). In such cases resolution of chronic candidiasis has occurred. However, the danger of a serious graft-versus-host (GVH) reaction is considerable. This is characterized by fever, a maculo-papular skin eruption, bloody diarrhoea and a Coombs-positive haemolytic anaemia. It may be observed in patients with a combined immuno-deficiency, and graft rejection usually occurs in cases where the immuno-deficiency is selective.

At the present time implants of foetal tissues appear to produce more successful results than bone marrow grafts. However, the overall success rate using any of these methods is still very low, so that the treatment of each case must be assessed on its own merits, considering that primary immunodeficiencies involving cell-mediated immune mechanisms are nearly always fatal within the first 2 years of life. A useful discussion of these indications is given in the *World Health Organization Memorandum on the Primary Immuno-deficiencies* (*Bulletin of the World Health Organization*, 1971).

Attempts have been made to avoid acute GVH reactions by separating stem cells from the immune competent cells, which were the mediators of the GVH reaction. The grafts were composed as far as possible of concentrated stem cells (Bekkum & Dicke, 1972). Using this technique, two out of ten patients with combined immuno-deficiency have survived for longer than 1 year, but delayed GVH reactions still occurred in a proportion of the patients.

Other observations have been reported in patients with Di George's syndrome, which includes congenital absence of the thymus, arising from a developmental defect of the third and fourth branchial pouches. The parathyroid glands are absent. Two patients who received grafts of foetal thymic tissue, subsequently developed delayed hypersensitivity reactions to a number of antigens, including candida monilia and dinitrochloro-benzene (DNCB). Both showed clinical improvement, including resolution of the chronic candidal lesions (August *et al.*, 1968; Cleveland *et al.*, 1968). However, rejection certainly occurred in one case, and probably in the other, and the immunological responsiveness declined after 18 months (*Bulletin of the World Health Organization*, 1971).

Another factor which must enhance the susceptibility to chronic oral candidiasis in patients with primary immuno-deficiencies is the necessity for repeated courses of antibiotics. Chronic candidiasis often appeared relatively late in the course of the disease and usually was not the presenting feature. It frequently gave rise to systemic infection, which required the administration of intravenous amphotericin B.

CHRONIC MUCO-CUTANEOUS CANDIDIASIS AS THE PREDOMINANT DISORDER. GROUP 2

Familial chronic muco-cutaneous candidiasis

Patients in this group have had local antifungal agents for many years, for example nystatin suspension or lozenges, amphotericin B lozenges and gentian violet. All of these were ineffective in clearing the lesions, which were usually confined to the mouth and nails. However, no other major clinical abnormality was apparent in these individuals and their general health was otherwise good.

Tests of cell-mediated immunity were abnormal in nine out of the seventeen patients in this group that we investigated, but were of more than one type and did not correlate precisely with the clinico-genetic classification (Higgs & Wells, 1972). Considerable fluctuation of *in vitro* lymphocyte function tests was observed in some individuals over a long-term period, although no change was noted in their clinical condition

In addition, a high incidence of latent iron deficiency was found in many patients. In the absence of anaemia, fifteen out of twenty individuals fully investigated were shown to have low serum iron levels and absent or reduced iron stores. One-third had evidence of reduced iron absorption, indicating the need for parenteral iron therapy.

Iron therapy. Considerable clinical improvement in the oral lesions was noted in nine out of eleven of our patients, when iron therapy was given in addition to their usual local antifungal agents, and some showed regeneration of normal nail when the iron therapy was continued for more than 6 months. Some of the individuals with familial chronic muco-cutaneous candidiasis were thought to have impaired iron absorption, because there was no significant rise in their serum iron level 4 h after a loading dose of oral ferrous fumarate (600 mg), even though evidence of iron deficiency had previously been established.

As a result of these findings, some patients were given a parenteral infusion of Imferon, the dose ranging from 10–20 ml according to their body weight. This was followed by oral iron in the form of ferrous fumarate (200 mg t.d.s.) for a period of 2–4 months. Most patients showed a significant improvement in the appearance of their oral lesions within that period of time. Three other patients, who were treated with oral iron only, took a longer time to shown an improvement and nail changes were not apparent for at least 6 months.

The effects of iron therapy included repapillation of the tongue, with reduction in ulceration and fissuring as well as loss of pain. The improvement was maintained as long as the iron therapy was continued but did not appear to correlate with the serum iron levels, which often remained low despite prolonged therapy. This unusual observation could not be accounted for in terms of a high percentage iron saturation, which is commonly seen in chronic inflammatory conditions and malignant disease, and this is now under further investigation.

Six of the eight patients in this group, who had negative skin tests to candida, developed a positive delayed hypersensitivity response after iron therapy and three out of six individuals showed changes in their abnormal *in vitro* lymphocyte function tests (Higgs, 1973). It is not clear at present if iron therapy directly affects cell-mediated immune responses or if the effect is indirect, mediated through a reduction in the antigen load. A report has been published recently showing depression of cell-mediated immune responses to tuberculin (purified protein derivative) and candida in iron-deficient individuals, which lends support to the former view (Joynson *et al.*, 1972). However, in patients with familial chronic muco-cutaneous candidiasis, clinical improvement was apparent with iron therapy, whether immunological abnormalities were corrected or not.

At present it is difficult to know what significance to ascribe to the role of iron metabolism in the aetiology of familial chronic muco-cutaneous candidiasis. Iron deficiency is commonly observed in the general population and is not usually associated with candidiasis. However, in this rare condition, we suggest that iron therapy might indirectly improve the quality of the oral epithelium, which could have an inherited structural or biochemical abnormality predisposing to the persistence of the pathogenic hyphal form of candida. Nevertheless the unusual difficulty experienced in correcting serum iron levels, even after prolonged therapy, may indicate a more fundamental defect related to iron metabolism.

Diffuse chronic muco-cutaneous candidiasis

Until recently amphotericin B was the only treatment for severely affected individuals, that is patients with DCMC (Buzdygan *et al.*, 1961). It is administered by slow intravenous infusion, because of poor absorption, and infusions, usually given on alternate days, often needing to be continued for several months. The initial dose of 0·25 mg/kg body weight is gradually increased to the optimal recommended dose of 1 mg/kg, which is given diluted in 5% dextrose. The dose may be increased to a maximum of 1·5 mg/kg if necessary. The major side effect is nephrotoxicity, and others include nausea, vomiting and local thrombophlebitis. Less common side effects have been described such as purpura, polymyositis and neuropathy (MacLean, 1962).

Immunological aspects. It has been shown recently that many of the affected individuals have variable defects in the expression of cell-mediated responses, which are probably less fundamental than those encountered in the primary immuno-deficiencies (Valdimarsson *et al.*, 1973). The defects encountered included reduced lymphocyte transformation *in vitro* to candida, which may result from the presence of an inhibitory serum factor and lack of migration inhibition factor (MIF) production in the presence of normal lymphocyte transformation. Attempts at immunological reconstitution, using similar methods to those employed in the primary immuno-deficiency diseases, have sometimes been successful and have occasionally resulted in dramatic clinical improvement.

HL-A compatible leukocytes. One patient treated in this way had chronic candidal oral and skin lesions, as well as widespread bronchiectasis. He was totally anergic to candida and DNCB and although his lymphocytes were capable of blastoid transformation to candida *in vitro*, he was unable to produced MIF (Valdimarsson *et al.*, 1972a). He was given a transfusion of HL-A compatible leukocytes from a healthy sibling and subsequently showed a gradual improvement. Later he was able to express delayed hypersensitivity reactions and produce MIF *in vitro*. There was a marked reduction in the frequency of his chest infections, weight gain and clearing of the oral, skin and nail lesions. This improvement has now been maintained for more than 2 years, suggesting that stem cells may have become established, giving rise to a new clone of thymus dependent lymphocytes capable of sensitization and MIF production.

Other investigators have attempted immunological reconstitution with some success, using in one case HL-A compatible marrow cells (Buckley *et al.*, 1968) and in another a foetal thymus graft (Levy *et al.*, 1971). These reports are relatively few and improvement has not often been maintained. The patient who received marrow cells later developed a graft-versus-host reaction which still constitutes a major problem when whole cell grafts are employed. Therefore it is particularly important to select patients very carefully for this procedure.

Transfer factor. Recently it has been possible to isolate from leukocytes an extract which is capable of conferring immunological sensitivity and is non-antigenic (Lawrence, 1970). The active principle is called transfer factor (TF) and is obtained by freezing, thawing and ultrasonication of leukocytes. The dialysable extract is concentrated and sterilized prior to subcutaneous injection. This procedure appears to be without side effects, but the beneficial effect is temporary and repeated injections are required at intervals of 2–4 months in order to maintain immunological sensitivity. It appears that a clear definition of the type of immunological defect may be necessary, because transfer factor is likely to be of benefit to those with reduced lymphocyte transformation to candida, rather than to individuals with normal transformation who do not produce MIF.

However, as the results of immunological reconstitution are at present generally unpredictable, because of the number of variable factors involved, much more work will be required before it will become possible to establish firm indications for the type of graft employed. For example, a patient from a different clinico-genetic group, that is an individual with the candida-endocrinopathy syndrome, reported by Levy *et al.* (1971), had chronic muco-cutaneous candidiasis in association with hypo-adrenocorticism, pernicious anaemia and a malabsorption syndrome. Lymphocyte transformation was normal to PHA but not to candida, and the child was anergic. Initial treatment with transfer factor on four occasions was unsuccessful, but subsequent grafts of thymus tissue, and later of foetal thymus and spleen, resulted in considerable clinical improvement, with some degree of restoration of immunological competence.

More recently the successful use of transfer factor was reported by Valdimarsson *et al.* (1972b) in a patient with diffuse chronic muco-cutaneous candidiasis, who appeared to have immunological abnormalities similar to those observed in Levy's patient. He had chronic oral and nail candidiasis as well as extensive crusted granulomas on his face and scalp. The patient's lymphocytes were able to produce MIF but transformation to candida was low, although there was no inhibitory serum factor. Considerable improvement was achieved, with clearing of the granulomas, but recurrence of lesions necessitated repeated injections of transfer factor at intervals of 3 months.

One group of investigators has used transfer factor combined with amphotericin B to reduce the antigen load as much as possible and the combination has proved to be effective (Editorial, 1971). Although there has been a striking improvement in a few cases, many others have shown little or no improvement, possibly because the concentration of extract was not great enough to produce a response (Fudenberg, 1972).

Immunological observations on patients treated with antifungal therapy. Not all severely affected individuals have defective cell-mediated responses, suggesting the existence of other significant aetiological factors (Higgs & Wells, 1972; Kirkpatrick, Rich & Bennett, 1971). The problem at the present time is to determine the true nature of these immunological defects, because immunological sensitivity may be restored following conventional therapy. Such a patient was described by Imperato, Buckley & Callaway (1968). He had diffuse chronic muco-cutaneous candidiasis with large candida granulomas and later developed a cryptococcal meningitis. He was deficient in IgA and totally anergic, although he had been able to produce a delayed hypersensitivity response in early childhood. He was also anaemic, so that parenteral iron was given as well as infusions of amphotericin B. Marked and lasting clinical improvement was reported, which included his nail infection, and he was subsequently able to produce a delayed hypersensitivity response to a number of antigens, including candida.

Restoration of immunological sensitivity after amphotericin B therapy has been reported on other occasions. For example the patient reported by Paterson *et al.* (1971) was shown to have a serum factor which inhibited cell-mediated immune responses. These observations suggested that some of the immunological defects encountered in these individuals were likely to be acquired and that immunological suppression, in the presence of a heavy antigen load, must be of some significance.

We have studied two patients with longstanding DCMC, both of whom showed similar immunological abnormalities. They had an inhibitory serum factor depressing lymphocyte transformation to candida, as well as negative delayed hypersensitivity skin responses to candida (Higgs & Wells, 1973b). They were also found to have chronic iron deficiency and low serum levels of vitamin A and pyridoxine.

One of these patients developed extensive granulomas on the scalp and these were treated by excision of the surface crusts, followed by topical application of a new antifungal agent, clotrimazole, combined with a simple keratolytic agent, that is 15% salicyclic acid in emulsifying ointment. Using this method the scalp was completely cleared of granulomas within 3 weeks. The other skin lesions were treated with topical Clotrimazole. The patient was given oral ferrous fumarate (200 mg t.d.s.)

for several months, and later this was supplemented with vitamin A and pyridoxine. Marked improvement of the oral and skin lesions rapidly occurred and there has been no recurrence of the granulomas after 15 months.

The second child was treated in the same way, again with substantial clinical improvement and similarly has remained almost clear of lesions for 2 years. However, both still have recurrent bacterial infections, for example chronic otitis externa and purulent blepharitis. Despite the administration of oral antibiotics for these infections, neither individual has had a recurrence of the oral candidiasis for more than a year.

The *in vitro* lymphocyte function tests were still abnormal in both, but one was able to express delayed hypersensitivity reactions to candida 6 months after starting therapy. Of further interest was the fact that despite a clear demonstration of iron absorption, continued iron therapy for more than a year corrected the iron deficiency anaemia, but the serum iron levels have remained low throughout the course of treatment. One child still had no iron stored in the marrow after 8 months of continuous iron therapy. In addition, after 6 months of pyridoxine and vitamin A therapy, there was no rise in the serum pyridoxine in one child, and a very poor response in the other. The latter also showed no rise in the serum vitamin A. The unusual difficulty experienced in replacing these deficiencies has also been observed by others, even after parenteral therapy (Levy *et al.*, 1971) and certainly warrants further investigation.

Since pyridoxine deficiency may influence both antibody production and cell-mediated immunity (Axelrod & Trakatellis, 1964) and vitamin A has a role to play in epithelial keratinization (Wolbach & Howe, 1933), deficiencies and metabolic abnormalities of this type are likely to be of significance in relation to the recurrence and chronicity of the disorder; a link between nutritional factors and the immune response may eventually be established.

The candida-endocrinopathy syndrome

The cause of the chronic candidiasis in this syndrome is obscure because it may develop several years before or after the endocrine manifestations, and endocrine replacement therapy generally does not improve the oral and nail lesions. Several hypotheses concerning the aetiology have been suggested, including auto-immunity (Hung, Migeon & Parrott, 1963), a defect in cell-mediated immunity (Kirkpatrick, Chandler & Schimke, 1970) and absence of a candida growth inhibition factor in the serum (Louria *et al.*, 1967). One patient with hypo-adrenocorticism and a macrocytic anaemia was given several injections of transfer factor without improvement but eventually responded to grafts of foetal thymus tissue (Levy *et al.*, 1971). Other patients with the syndrome showed little response to cell grafts (Kirkpatrick *et al.*, 1970) and several more have been observed, in whom it was not possible to demonstrate any defect of cell-mediated immunity (Valdimarsson *et al.*, 1973).

Local antifungal agents generally control the candidiasis, which is fortunately relatively mild in most patients. However, iron deficiency may be a feature of patients with different types of endocrinopathy. In our own series, in addition to two patients with hypoparathyroidism and hypoadrenocorticism, one was a diabetic, a second had thyrotoxicosis and a third was myxoedematous and subsequently developed hypoparathyroidism. After combined parenteral and oral iron therapy, the patient who was thyrotoxic showed complete clearance of the chronic oral and nail candidiasis even when her thyrotoxicosis was still untreated, and the candidiasis has not relapsed 2 years later. The patient with myxoedema and hypoparathyroidism had an iron deficiency anaemia but was not able to absorb iron; parenteral iron therapy was therefore indicated.

There is some evidence to suggest that endocrine dysfunction of different types may have a common inhibitory effect on gastric acid secretion, ranging from a mild disturbance to complete achlorhydria (Donegan & Spiro, 1960; Kirsner, 1953). This may have a significant role to play in the development of impaired iron absorption in these patients, and may not always be fully reversible following endocrine replacement therapy.

Patients who do not have the candida-endocrinopathy syndrome, but who have isolated endocrine abnormalities such as thyrotoxicosis or diabetes mellitus, often have chronic candidiasis as a complication, but this may improve when the underlying disorder is treated. Diabetics, whose disease is poorly controlled, have been shown to have a significant decrease in polymorphonuclear phagocytosis, which is reversible and which leads to a reduction in effective bacterial killing (Bagdade, Nielson & Bulger, 1972).

Late-onset chronic muco-cutaneous candidiasis

Nutritional deficiencies may also have a significant part to play in the aetiology of late onset chronic muco-cutaneous candidiasis. For example, one patient aged 71 was reported by Ephraim in 1949 to have chronic oral candidiasis, but otherwise had good general health. He showed no response to several vitamins given parenterally, but his lesions cleared completely on large doses of vitamin A.

Iron deficiency was often found in this group of patients and improvement has been observed after iron therapy (Higgs & Wells, 1972). It was extremely important to try and elucidate the cause of the iron deficiency in these individuals, because several cases have been reported of relatively long duration in which an underlying neoplasm was eventually detected, often of an unusual type (e.g. Rothman, 1959; Engman & Weiss, 1920). Chronic candidiasis associated with iron deficiency may represent an early sign of serious underlying organic disease.

Late-onset chronic oral candidiasis has also been reported in patients with a denture stomatitis (Rose, 1968) and latent iron deficiency was a prominent feature of this report. The condition arises as a result of a large number of yeasts being harboured under the dental plate (Cawson, 1969). The lesions are usually confined to pressure areas, for example the hard palate. This type of infection is often very difficult to eradicate and strict attention to oral hygiene is of great importance. Denture stomatitis can usually be controlled by local antifungal agents, for example nystatin, but further improvement may be obtained if iron therapy is given in addition for several months.

Cutaneous candidiasis occurring in this late-onset group is very rare and is often associated with the syndrome of thymoma, myopathy and muco-cutaneous candidiasis. The thymoma is generally malignant and treatment depends on the stage of the tumour growth. Skin lesions do not resolve when the tumour is treated and can only be controlled with local antifungal agents. Patients usually succumb within 5 years as a result of the malignant condition (Maize & Lynch, 1972).

NEW ANTIFUNGAL AGENTS

Several new antifungal agents have been brought into use during the last five years in particular 5-fluorocytosine (Roche) and Clotrimazole (Bayer Pharmaceuticals). These differ from the polyene antibiotics in that they are both synthetic substances and their therapeutic effects are still being fully assessed.

5-*Fluorocytosine* is an anti-metabolite of some fungi, and is active against several species including *Candida albicans*, the Aspergillus species and *Cryptococcus neoformans*. It may be given systemically and the recommended dose for adults and children is 100 mg/kg daily for a period of 4 weeks. It is also available in the form of a topical application. 5-Fluorocytosine has been reported to be effective in the treatment of systemic candidiasis (Tassel & Madoff, 1968) and may sometimes produce improvement in recurrent muco-cutaneous candidiasis (Louria, 1969).

We were unable to demonstrate any lasting improvement in four out of five individuals with familial chronic muco-cutaneous candidiasis who received systemic therapy (100 mg/kg body weight), and this had to be discontinued in another patient because he developed a neutropenia and a generalized macular eruption. In addition, one other individual, who had the candida-endocrinopathy syndrome, developed a severe relapse in his oral lesions 4 weeks after starting systemic therapy. It is possible that this may have been due to the development of drug resistance, which had previously been described in earlier reports (Utz *et al.*, 1969). These authors also referred to a significant incidence of relapse in the patients that they studied. The problem of drug resistance is of importance in the choice of antifungal agents used, particularly as this is not usually a feature of treatment with polyene antibiotics such as amphotericin B. Nevertheless most of the patients in our study were unresponsive to polyene antibiotics, that is nystatin and amphotericin B, when used topically indicating that these observations may be confined to the specialized group of patients studied.

Clotrimazole. This is another synthetic compound (bis-phenyl-(2-chlorophenyl)-1-imidazolyl methane) which is a trityl-imidazole derivative and exhibits a wide spectrum of fungistatic activity in therapeutic doses. It is active against many species of Candida, dermatophytes of the genera Trichophyton and Microsporon and other budding fungi, including *Coccidioides immitis*, *Histoplasma capsulatum* and *Cryptococcus neoformans* (Plempel *et al.*, 1969). The dosage is dependent on the sensitivity of the causative micro-organism, but is usually in the region of 60 mg/kg body weight daily for adults, taken in three divided doses. In children the dose may vary between 60 and 150 mg/kg body weight depending on the sensitivity of the micro-organism and the severity of the condition. The optimal duration of treatment as reported by Plempel *et al.* (1969) was found to be 8–12 days in systemic mycosis and 12–16 days in dermatomycosis. Shorter periods of treatment were not advised because the fungistatic property of the compound involved the risk of relapse and the possibility of developing resistance.

We have observed a high incidence of side effects in patients with FCMC treated with systemic Clotrimazole. In particular there was a marked psychological disturbance in some patients. Therefore we were unable to continue therapy for any length of time, but in two individuals, who continued systemic therapy for three months, only slight improvement was apparent (Wells *et al.*, 1972).

Two patients with DCMC were treated initially with systemic Clotrimazole, but a rise in liver transaminases was observed in both individuals and one subsequently developed a reversible neutropenia. However, extremely good results were later obtained in both the patients using topical Clotrimazole (Higgs & Wells, 1973b). Other workers have reported considerable improvement in the oral lesions of some patients treated with systemic Clotrimazole without the occurrence of side effects (Meinhof & Günther, 1972).

AMPHOTERICIN B IN SMALL DOSES

Another interesting observation has recently been reported concerning the effectiveness of amphotericin B given parenterally in small amounts. It was said that a total dose of 10–355 mg given intravenously over 4–18 days was as effective in clearing chronic candida lesions as the conventional dose of 1 mg/kg, and there were no toxic side effects (Medoff *et al.*, 1972). Fourteen patients were described, of whom ten had acquired candidiasis after receiving antibiotics. One patient had had chronic oral candidiasis since childhood and appeared to have similar clinical features to our patients with FCMC. She had previously developed toxic effects following conventional amphotericin B treatment and her lesions eventually recurred. She was given 35 mg of amphotericin intravenously during a 4 day period and remained free of oral candidiasis for a year. This approach may prove to be extremely valuable in treating patients with chronic disease who would not be considered suitable for immuno-therapy.

CONCLUSION

Chronic muco-cutaneous candidiasis has long been one of the most difficult therapeutic problems with which the dermatologist may be confronted. It is gratifying that new approaches to treatment are becoming available. However, their correct use depends on an accurate clinical assessment, and a clear distinction must be made between the different types of immunological abnormalities that may be encountered.

In those patients in whom the lesions are confined to the mouth and nails, it would appear to be as important to correct any underlying deficiency states, in particular iron deficiency, as it is to give an effective antifungal agent. It is likely that a course of low dose intravenous amphotericin B combined with parenteral and prolonged oral iron therapy may prove to be a useful routine treatment and that immunological abnormalities occurring in these patients may be corrected spontaneously.

In patients who are more severely affected, that is with a primary immuno-deficiency or diffuse chronic muco-cutaneous candidiasis, improvement may sometimes be achieved by immunological reconstitution. However, it is necessary to employ the appropriate techniques in each case, because different immunological defects appear to require specific types of graft which are not freely interchangeable. The use of combined therapy, that is an effective antifungal agent, replacement of nutritional deficiencies and immunological reconstitution has yet to be assessed, but it is likely that better long-term therapeutic results will eventually be achieved by using this approach.

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Intradermal triamcinolone treatment of nail dystrophies

E. ABELL AND P. D. SAMMAN

St John's Hospital for the Diseases of the Skin, Lisle Street, London, W.C.2.

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SUMMARY

With a needleless injector (Port-O-Jet), triamcinolone acetonide can readily be injected into the area of the finger-nail matrix, through the posterior nail fold. 100 cases of various nail dystrophies have been treated in this way.

Dystrophies involving the nail matrix have been found to resolve substantially in the majority of cases. Onycholysis was found to improve occasionally, but generally relapsed rapidly. However, when onycholysis was associated with matrix abnormalities more consistent and prolonged remissions of the onycholytic areas occurred.

That fluorinated corticosteroids injected into the posterior nail fold could induce remission of dystrophic nails in psoriasis and lichen planus was first demonstrated by Gerstein (1962). Using a needle and syringe, local anaesthetic was required, but in more than half his cases satisfactory improvement occurred. With the development of needleless injectors it became possible to perform this type of treatment quickly and with little discomfort. In a small series reported earlier (Abell, 1972), this technique produced remission in nearly 80% of treated nails. This study reports the results of treatment of nail dystrophies, including psoriasis, lichen planus, oncycholysis, alopecia areata, split nails and mucous cysts, over a 2-year period.

MATERIAL AND METHODS

One hundred patients with nail dystrophies as described below were treated by injections of triamcinolone acetonide at a concentration of 5 mg/ml on three occasions at 2–4-weekly intervals. Severely dystrophic nails were subsequently given intermittent injections at 6–10-weekly intervals, as deemed necessary. Deep intradermal infiltration of the posterior nail fold was achieved using the Port-O-Jet injector, one to four injections of 0·1 ml being required for each affected finger or toe nail. A total of 693 nails was treated. Using a 45% solution of Hypaque in the same way, the extent of penetration can be shown radiographically (Fig. 1) to be confined to the nail fold.

Sterilization procedures for the apparatus, using 0·5% chlorhexidine in 70% industrial spirit, have been previously described (Verbov & Abell, 1970). To simplify analysis of results, the patients were grouped under the following headings.

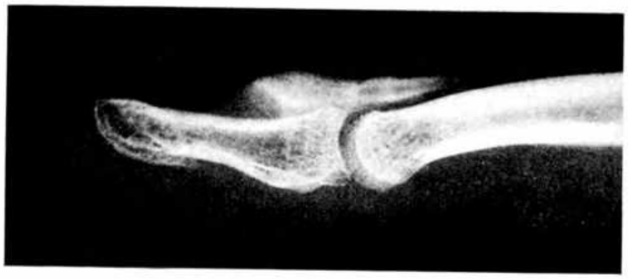

FIGURE 1. X-ray showing Hypaque infiltration of posterior nail fold.

RESULTS

The results are shown in Table 1.

Psoriasis, involving the matrix only

Twenty-four patients (sixteen male and eight female) of average age 41 years (range 6–70) with matrix involvement, from mild pitting and ridging to gross destruction of the nail plate, were treated. The average duration of nail changes was 4 years (range 0·5–15), and seven also had cutaneous psoriasis.

Twenty-one patients (84%) showed improvement (Fig. 2), normal or nearly normal nail regrowth occurring in nineteen. No change was seen in three patients, all of which had very severe destructive nail involvement, two having persistent pustulation of the nail bed. Within the 2-year period, ten relapses had occurred after a mean duration of 6·2 months.

TABLE 1. Results of intradermal injection of nail dystrophy with triamcinolone acetonide

Dystrophy	No. of cases	Result in the matrix	Result in onycholysis	Follow-up over 2 years
Matrix only	24	19 normal/greatly improved 2 improved 3 no change		10 relapses at 3–12 months, mean 6·2 months
Combined matrix and onycholysis	20	17 normal/greatly improved 2 improved 1 no change	9 cleared/greatly improved 5 improved 6 no change	4 matrix relapses 6–12 months, mean 8·5 months 8 onycholytic relapses at 4–6 months, mean 4·5 months
Psoriatic onycholysis	14		3 cleared/greatly improved 4 improved 7 no change	4 relapses at 3–4 months
Idiopathic onycholysis	15		3 greatly improved 4 improved 8 no change	7 relapses at 3–9 months, mean 5 months
Lichen planus	11	7 normal/greatly improved 1 improved 3 no change		2 relapses at 8 and 12 months

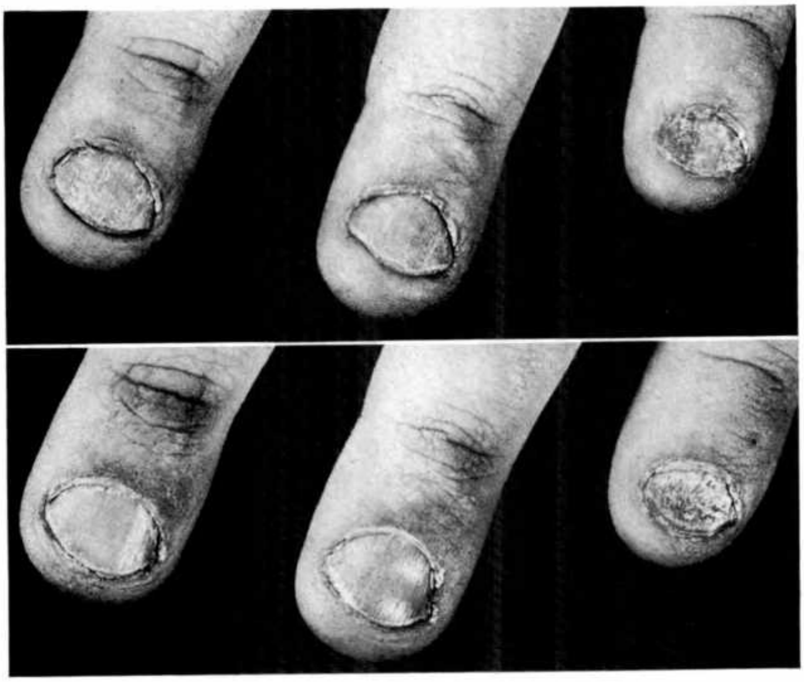

FIGURE 2. Psoriatic nail dystrophy before and 10 weeks after treatment of the third and fourth nails.

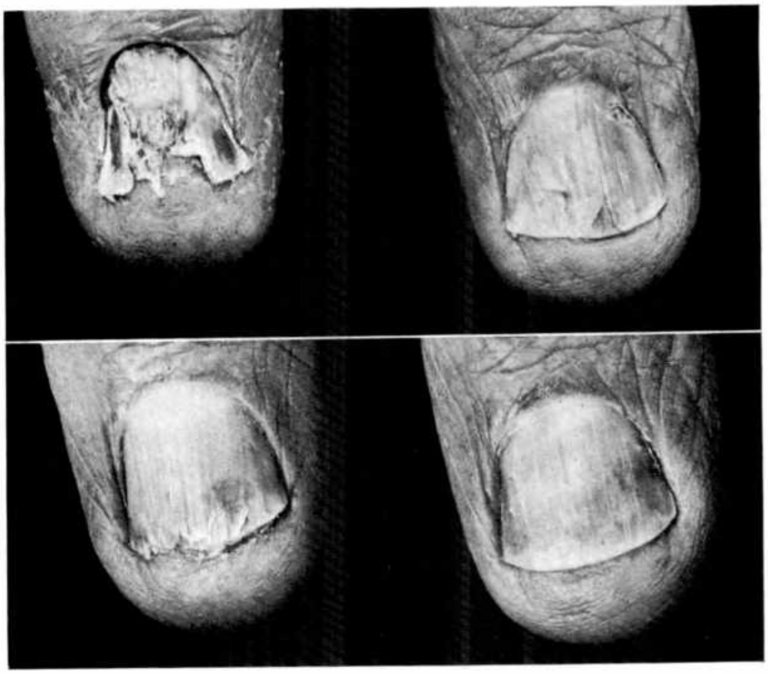

FIGURE 3. Psoriatic matrix dystrophy with onycholysis before and 9 months after intermittent treatment.

Psoriasis, combined matrix involvement and onycholysis

Twenty patients (eight male and twelve female) of average age 42 years (range 20–61) demonstrated, in addition to matrix changes, varying degrees of onycholysis. The average duration of involvement was 5 years (range 0·5–15); thirteen patients had cutaneous psoriasis and four had distal interphalangeal joint arthritis.

In this group matrix changes responded well (Fig. 3), nineteen (95%) showing improvement, and relapses were rather less frequent and appeared later. Fourteen patients (70%) showed some resolution of the onycholysis. Seventeen patients in this group had either a moderate or slight degree of onycholysis, and half had useful improvement, with only two completely clearing. In the remaining three onycholysis was severe and unresponsive.

Onycholysis associated with psoriasis

Fourteen male patients with onycholysis also had other features suggesting a psoriatic association. Thirteen had cutaneous psoriasis and five psoriatic arthropathy. The average age of the patients was 49 years (range 31–64) and the average duration of the nail involvement was 4 years (range 0·25–25).

In three patients complete resolution of onycholysis followed injection, and one remained in remission for a year. Otherwise improvement was slight, short lived, and infrequent.

Idiopathic onycholysis

After excluding other causes for onycholysis, fifteen cases (five male and ten female) with idiopathic onycholysis of average duration 7·5 years (range 0·5–20) were treated. Their average age was 46·5 years (25–70).

Three cases showed evidence of improvement, but no patient achieved full clearance, and relapse was relatively rapid.

Lichen planus

Eleven cases (nine male and two female) showing lichen planus involving the nails, from longitudinal ridging to severe destructive dystrophy, pterygium formation and nail shedding, were included. Their average age was 34 years (range 7–63) and duration of nail involvement averaged 3 years (range 0·5–12). Five cases had cutaneous lichen planus, and in four, nail biopsies using Zaias' technique (1967) supported this diagnosis.

Seven patients showed complete or nearly complete resolution (Fig. 4), and three cases failed to respond. One of these cases, with the isolated destructive nail dystrophy described by Marks & Samman (1972), developed progressive involvement and subsequent loss of all the finger nails, repeated injection having no apparent effect upon the process. Pterygium for 1 year and established ridge formation for 12 years were the pretreatment conditions of the two other cases showing no response. Relapses in two patients occurred at 8 and 12 months.

Miscellaneous group

This group included sixteen cases as detailed in Table 2.

In three of the four patients with alopecia areata the nail dystrophy was limited either to uniform pitting or to coarse ridging, and in all these full resolution occurred. Within 6 months two had shown some return of the dystrophy. The patient showing no improvement had gross destructive nail changes associated with alopecia universalis of 6 years' duration. Alopecia totalis of 6 months' duration was a feature of two of the cases showing resolution, and the third had recurrent alopecia areata of the scalp for 6 years.

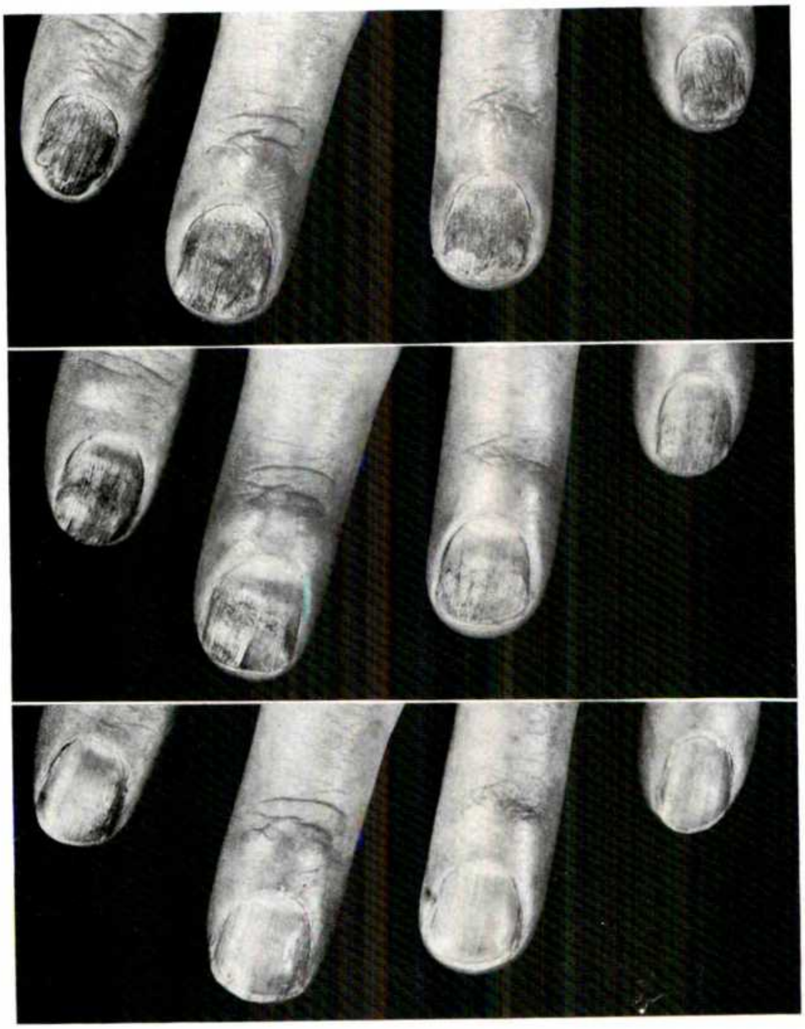

FIGURE 4. Lichen planus before, 9 weeks, and 1 year after treatment.

TABLE 2. Intradermal triamcinolone acetonide in nail dystrophy

Condition	No. of cases	Results
Alopecia areata	4	3 fully resolved, 2 relapsed at 4 and 6 months
Split nails	3	2 fully reformed
Mucous cysts	6	5 cysts resolved but relapsed rapidly 1 resolved without relapse at 6 months
Traumatic	1	Slight improvement of nail plate
Pityriasis rubra pilaris	1	No change
Sarcoidosis	1	No change

Two split nails, one following 'whitlow' infection and one following trauma (4 years), reunited after injections. A traumatic split nail of 10 years' duration was unaltered.

Mucous cysts also resolved after injection, but returned rapidly afterwards, with one exception. All cases were treated because of 'furrowing' of the nail plate, and although only one case made a complete recovery some improvement in the furrow was usual.

Single cases of traumatic damage, pityriasis rubra pilaris and sarcoidosis were marginally improved or unchanged.

Complications

Few problems arose from this type of treatment. Haemorrhage at injection sites may easily be controlled by pressure. Sepsis was not seen, and although atrophy occurred it was usually slight and transient, and did not persist, even in those treated intermittently over 2 years. X-ray examination of the digits was performed at the end of the first and second years in all patients receiving intermittent injections. No radiographic evidence of osteoporosis was seen. On three occasions penetration of the nail plate within the posterior nail fold occurred. The triamcinolone suspension was seen exactly to fill the area of the lunule. Nail growth progressed uninterrupted, but incorporation into the nail plate of a white and red transverse band followed.

DISCUSSION

This study supports the findings of the preliminary series. Triamcinolone acetonide injections induce a remission of psoriatic matrix dystrophy in a high proportion of cases, and the same good response also occurs in lichen planus and alopecia areata. Dystrophies affecting toe nails have also been treated with similar results, but in adult patients treatment was required for a longer period of time and the resolution was very slow. Severely destructive matrix abnormalities in all three dystrophies usually failed to improve, and persistant pustular psoriatic changes were unaffected.

Relapse in all these conditions must be anticipated, but the periods of remission have been sufficiently long for retreatment or intermittent therapy to be a practical procedure. Frequently relapse was milder than the original state and involved fewer nails.

Where onycholysis co-existed with matrix psoriatic changes approximately 50% improved or cleared completely. Onycholysis alone, whether of psoriatic or unknown origin, was not consistently improved, and it is possible that spontaneous resolution may have accounted for these findings. It was not found possible to perform injection directly beneath the onycholytic areas.

Injection may be useful in traumatic split nails, but it infrequently produced worthwhile resolution of mucous cysts.

That corticosteroids are responsible for the matrix remission is suggested by the regular and consistent way in which the dystrophy clears (Fig. 4) and the absence of spontaneous remission in any untreated nails (Fig. 2). Untreated normal nails have frequently been seen to become involved while treated nails resolve.

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Hydroxyurea and macrocytosis

STANLEY SPIER, LAWRENCE M. SOLOMON, NANCY B. ESTERLY
AND WALTER FRIED

Departments of Dermatology and Medicine, Abraham Lincoln School of Medicine, University of Illinois,
P.O. Box 6998, Chicago 60680, Illinois, U.S.A.

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SUMMARY

Nine patients who were treated for psoriasis with hydroxyurea displayed peripheral macrocytosis. Mild megaloblastic changes were also seen in the bone marrow of three patients. One patient receiving hydroxyurea developed mild anaemia, another leukopenia. Macrocytosis did not appear to be secondary to an alteration in serum B_{12} or folate levels. The haematological side-effects of hydroxyurea therapy, at the relatively low doses used to treat psoriasis, are similar to those seen with the higher doses used for the treatment of malignant tumours or leukaemia.

Hydroxyurea has gained increased interest as a systemic agent for the treatment of psoriasis. One reason for this is the possibility that it may have fewer undesirable side-effects than methotrexate. Although it has been known that hydroxyurea administered in adequate dosage for the treatment of psoriasis may produce bone marrow depression and resultant leukopenia (Rosten, 1971), other changes have received little attention.

This paper stresses another haematological effect of hydroxyurea, namely megaloblastic changes in the bone marrow with macrocytosis. Hydroxyurea used in dosages adequate to control neoplastic processes may produce megaloblastic marrow changes and macrocytosis (Kennedy & Yarbro, 1966). The authors (Spier *et al.*, 1971) and Touraine, Revuz & Tulliez (1972) have reported similar changes resulting from the dosage levels used to treat psoriasis. This report amplifies our previously reported studies in nine patients under hydroxyurea therapy for psoriasis.

SUBJECTS AND METHODS

The subjects were seven men and two women, all Caucasoids, ranging in age from 28 to 60 years. Eight of the patients were in good health other than having psoriasis. One patient, a 31-year-old man (patient 2), developed two myocardial infarctions while hospitalized for psoriasis and before treatment with hydroxyurea.

Seven of the patients had previously received methotrexate. Of these, four (patients 2, 5, 7 and 8) had not had methotrexate for at least 4 months. Of the remaining three, two (patients 1 and 4) had been taking methotrexate until 1 week before and patient 6, until 3 weeks before hydroxyurea was started.

In seven patients 500 mg of hydroxyurea was given orally twice daily, with no subsequent changes in dosage. Patient 6 received 1·25 g daily for 11 weeks and patient 5 received 750 mg daily for one month. Except for these periods these patients were receiving 500 mg twice daily.

Haematological studies

Bone marrow aspirations were performed on patients 1, 6, 7 and 8. Patient 7 had a bone marrow study 4 days before initiation of hydroxyurea therapy. The remaining three patients had bone marrow aspirations performed during therapy. In addition, patient 1 had a bone marrow study 7 months after hydroxyurea was discontinued.

Serum folate was measured by a microbiological assay, using *Lactobacillus casei*. This assay detects only the reduced forms of folic acid in the serum. The predominant serum folate detected by this method is thought to be 5-methyltetrahydrofolate. Serum B_{12} levels were measured by a modification of the radio-isotope dilution method of Lau *et al.* (1965).

All patients had mean corpuscular volumes (MCV), white blood counts, platelet counts and haemoglobins measured repeatedly at intervals of approximately 2 weeks.

RESULTS

Fig. 1 demonstrates the MCV values in five patients who had received hydroxyurea without interruption. Fig. 2 records the mean corpuscular volumes of the four patients who received hydroxyurea discontinuously. Duration of treatment ranged from 2½ to 10 months. In all nine patients topical medications were used in addition to hydroxyurea.

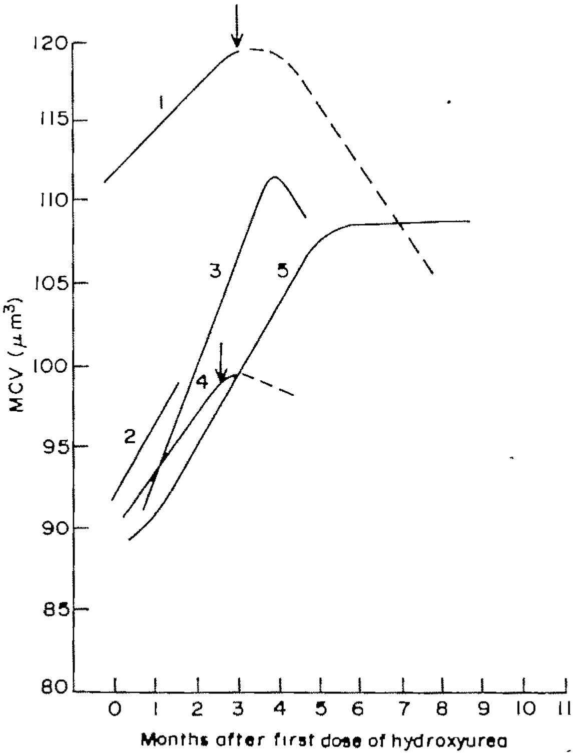

FIGURE 1. MCV response to continuous hydroxyurea therapy. ———, Patient on medication; - - - - -, patient off medication; ↓, hydroxyurea stopped.

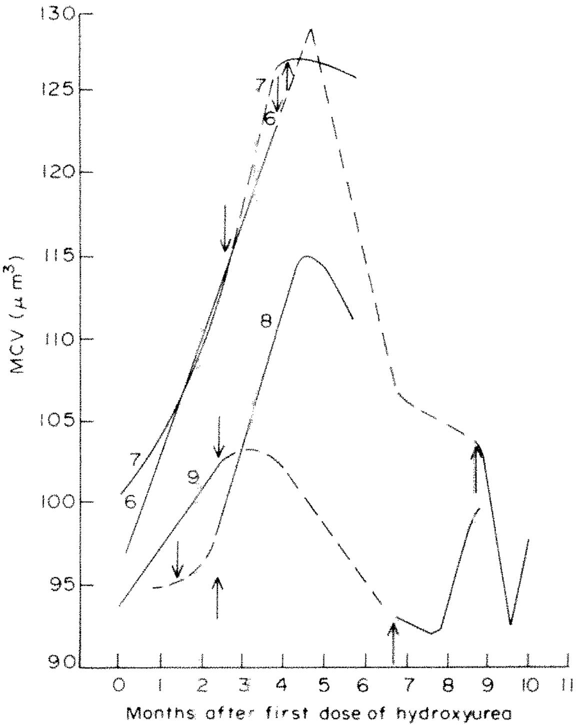

FIGURE 2. MCV response to discontinuous hydroxyurea therapy. ———, Patient on medication; - - - - -, patient off medication; ↑ hydroxyurea restarted; ↓, hydroxyurea stopped.

The MCV of each of the nine patients rose after treatment with hydroxyurea, indicating the presence of macrocytosis.

Patient 1 developed macrocytosis following initiation of hydroxyurea; after the drug was discontinued the MCV steadily decreased. The same pattern was found in patient 4, even though he received methotrexate immediately after hydroxyurea was discontinued. The MCV of patient 3 showed a marked increase following initiation of hydroxyurea therapy. The MCV had begun to decline, however, before treatment was stopped. Patient 5 demonstrated an initial increase in MCV on hydroxyurea, but did not increase beyond 100 μm^3, although therapy was continued. Patient 2 displayed a steady increase of MCV soon after initiation of hydroxyurea therapy.

Patient 6, immediately after starting hydroxyurea, had a steady increase in his MCV. Following discontinuation of therapy the MCV decreased. When hydroxyurea was reinstituted the MCV continued to decrease, but then it once more reversed direction. A similar decrease in MCV was seen in patient 7. Patient 8 displayed a pattern of increasing MCV during a second course of medication (initial treatment was of short duration), but just as with patient 3, the MCV had begun to decline before the hydroxyurea was stopped. Patient 9 showed an MCV climbing while receiving hydroxyurea, falling after the medication was stopped and increasing after hydroxyurea was reinstituted.

Table 1 lists the findings in the bone marrow aspirations. Those patients who had had marrow aspirations while on hydroxyurea all showed megaloblastic changes. In contrast, no such changes were noted in the two patients who were untreated at the time of the aspiration.

Table 2 demonstrates the serum folate levels prior to, during, and following hydroxyurea therapy.

TABLE 1. Bone marrow studies

Patient	Bone marrow	Relation to hydroxyurea therapy
7	Normal	Before
1	Normal	After (7 months)
6	Mild megaloblastosis	During (4 months)
1	Mild megaloblastosis	During (3 months)
8	Mild megaloblastosis	During (3 months)*

* Second course of therapy.

TABLE 2. Serum folate levels

Hydroxyurea therapy	Values	No. of determinations	Range (ng/ml)
Before	Normal	3	5–7 8
	Low	1*	2
During	Normal	10	5–16
	Low	2	2–4
After	Normal	2	5–16
	Low	3	2·8–3

* Patient had received methotrexate.

In most instances the folate levels were normal. The one low folate level obtained prior to hydroxyurea therapy may have been due to a previous course of methotrexate. Two low folate levels were recorded in patients on hydroxyurea; one was found in the patient who had received methotrexate (patient 4) and the other in a patient (patient 6) who had received hydroxyurea for 12 weeks. Hydroxyurea had been discontinued and then reinstituted in the latter patient. 2 and 3 weeks after the drug was restarted, the folate levels were found to be within normal limits.

Patient 1 developed low folate levels 6, 9 and 12 weeks after hydroxyurea was discontinued. Two weeks before starting treatment she had had normal levels of serum folate, and after receiving hydroxyurea for 7 weeks her serum folate was normal.

TABLE 3. Serum B_{12} levels

Patient	Serum B_{12} (pg/ml) (Normal, 200–800 pg/ml)	Relation to hydroxyurea therapy
4	210	Before
9	224	After (4 days)
1	660	During (4 weeks)
6	270	During (16 weeks)
6	408	During (2 weeks)*
8	520	During (8 weeks)*

* Second course of therapy.

Hydroxyurea does not appear to depress the serum level of vitamin B_{12} (Table 3).

In eight of the nine patients there was no detectable depression of the white blood count during or following hydroxyurea therapy. Patient 7 had persistent leukopenia (3000–4800 WBC/mm^3) over a 4-month period. In this patient leukopenia was noted 2 months after medication was started; the previous white cell counts had been normal.

Only one of the patients developed anaemia. The haemoglobin decreased from 15·8 g/100 ml to 12·0 g/100 ml over a 2½ month period, but returned to normal after drug therapy was discontinued. After a few months hydroxyurea was restarted, and he again exhibited a mild anaemia.

In all nine patients there was no thrombocytopenia throughout the course of therapy.

Six of the nine patients showed substantial clinical improvement in their psoriasis while on the hydroxyurea.

DISCUSSION

Pharmacological properties of hydroxyurea

Hydroxyurea was synthesized by Dresler & Stein (1869). Its empirical formula in $CH_4N_2O_2$ and its structural formula is: NH_2–CO–NH–OH.

It was first employed in biological studies by Rosenthal, Wislicki & Kolleck (1928), who demonstrated that, in rabbits, the drug produced megaloblastic changes similar to those seen in pernicious anaemia, as well as depression of leukocyte formation. In 1960, antitumour activity was reported in animal screening studies (Stock, Clarke & Philips, 1960). Subsequently, hydroxyurea was demonstrated to have a depressive effect on the bone marrow and to cause megaloblastosis in the marrow of humans, although there was objective improvement in a variety of solid tumours and in acute and chronic leukaemia (Kennedy & Yarbro, 1966).

Hydroxyurea interferes with DNA synthesis by an incompletely understood mechanism, without influencing the synthesis of RNA or protein (Morse, Rencricca & Stohlman, 1969). Krakoff, Brown & Reichard (1968) showed that hydroxyurea prevented the conversion of ribonucleotides to deoxyribonucleotides by interference with the action of the enzyme ribonucleoside diphosphate reductase. However, this *per se* does not explain all of the effects of hydroxyurea on DNA synthesis. The inhibition of DNA synthesis by hydroxyurea cannot be corrected by administration of deoxyribunocleotide (Yarbro, 1968). In addition, Vogler, Horowitz & Groth (1969) showed that hydroxyurea exerted an inhibitory effect on orotic aciduria which did not correlate with its effect on ribonucleoside diphosphate reductase.

Since hydroxyurea has been found to be selectively toxic to cells during the S-phase of the cell cycle (Hennings & Devik, 1971), its effect in psoriasis may be to decrease cell turnover by inhibition of DNA synthesis in the epidermal cells. Megaloblastosis is considered to be the result of impaired synthesis of DNA in the face of permissive or continuing synthesis of RNA. The result is a large cell with an increased net amount of RNA (Kennedy & Yarbro, 1965).

The dosage of hydroxyurea used in the treatment of tumours and leukaemia is higher than that currently in use for the treatment of psoriasis. In one study, 40 mg/kg/day was taken orally by twenty-three patients. Subsequent examination of the bone marrow of thirteen of these patients on days 3 and 5 of therapy showed megaloblastic changes in all of them (Bergsagel *et al.*, 1964). 40 mg/kg/day taken orally caused bone marrow depression (requiring cessation of therapy) in at least 70% of the patients within 4 to 22 days. The cells of the myeloid series were the first to be affected; neutropenia was seen by day 10 in most of them. Thrombocytopenia was observed in about 20% of the patients. Anaemia developed slowly and became a marked feature of the erythropoietic toxicity in 34% of the patients.

With oral doses of 60 and 80 mg/kg/day, marrow toxicity was observed earlier and in a greater percentage of cases, but the pattern was similar to that described above (Bergsagel *et al.*, 1964).

Findings in our patients

In our study, the dosage of hydroxyurea used as treatment for psoriasis was substantially lower than that required to treat malignant tumours or leukaemia, but, nevertheless, resulted in mild megaloblastic changes in the bone marrow of three patients. Two patients, while off medication, did not show megaloblastic changes in their marrow. However, peripheral macrocytosis was found in all of our patients in association with hydroxyurea therapy.

Only one of our patients developed mild anaemia, possibly due to hydroxyurea. After discontinuation of the medication, the haemoglobin concentration returned to normal and, on reinstitution of therapy, there was a recurrence of mild anaemia. Only one of the nine patients developed leukopenia, and this was also presumably associated with hydroxyurea therapy. None of our patients developed thrombocytopenia.

It would be of interest to know whether or not these changes can be prevented by the use of vitamin B_{12} or folic acid. Thurman & Watkins (1964) showed that serum B_{12} and folate levels were normal in eighteen children treated with 40 mg/kg/day of hydroxyurea orally. Megaloblastosis occurred despite treatment with vitamin B_{12} or folic acid or both, before and during hydroxyurea therapy. Therefore megaloblastosis most probably was not due to deficiency of folate or B_{12}.

None of our patients whose B_{12} levels were studied during or shortly after hydroxyurea therapy showed a deficiency of this vitamin. Only two of the six patients who had had serum folate levels determined while on hydroxyurea showed a folate deficiency; patient 6 in one of three determinations and patient 4 in one of four determinations. These low folate values as well as those in patient 1 occurring after cessation of hydroxyurea may have been due to a laboratory error, malabsorption secondary to psoriasis (Summerly & Giles, 1971) or other unknown factors. With the relatively low doses of hydroxyurea used for psoriasis, altered folate and B_{12} levels are apparently not a major factor in causing macrocytosis. This is consistent with previous observations that macrocytosis produced by larger doses of hydroxyurea used for tumours and leukaemia is not associated with folate or B_{12} deficiency.

Hydroxyurea appears to be a useful medication for treatment of severe, widespread, recalcitrant psoriasis in some patients. The haematological side effects of hydroxyurea therapy at 1 g/day are similar in kind to those seen at higher doses. In most instances, these side-effects do not appear to be associated with anaemia, and the megaloblastic and macrocytic changes appear to be reversible.

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Comment

TRACE ELEMENTS AND SKIN PIGMENTATION

Of the many natural pigments displayed by human skin, melanin, if not always the most prominent, is perhaps the most significant. Its formation in the skin has been well documented over the last 25 years, yet, because of the difficulties in isolating it in a pure state, its chemical structure and composition remain largely unknown (Duchon, Fitzpatrick & Seiji, 1968). Likewise, there is little known about the interactions between melanin and that group of elements, found in the body in a concentration of less than 100 p.p.m., which are becoming increasingly known as 'trace elements'.

In animals, efforts to achieve a better understanding of these interactions seem to have been prompted by the economic value attached to wool, fur and other coating materials. The best known example is that of copper and its role in wool pigmentation. The loss of pigment found in copper deficiency states in sheep has been attributed to a breakdown in the earlier stages of melanin synthesis, which are catalyzed by the copper-dependent enzyme tyrosinase (Underwood, 1971). On the other hand, colour changes of hair found in children with a protein deficient state were not related to their copper nutritional status (Gopalan, Reddy & Mohan, 1963).

Of the fifty or more trace elements known to exist in the body, zinc offers the most interesting prospect in its relation with melanin. Very high zinc levels are found in heavily melanised structures, like the choroid of the eye (Underwood, 1971), pigmented moles (Molokhia & Portnoy, 1973) and melanosomes (Seiji *et al.*, 1963). The relatively minute mass of melanin present in these tissues makes it safe to conclude that this pigment must be extremely rich in zinc.

However, the nature of the association between zinc and melanin remains unexplored and the significance of such a high zinc content in pigmented tissues is at present unknown. It has been suggested that melanin may be acting as a potent chelating agent, binding trace metals like zinc (Lorincz, 1954). *In vitro* studies, however, have shown that melanin behaves more like a weak cation exchanger (Bruenger, Stover & Atherton, 1967). The possibility that zinc may be catalyzing some of the later steps of melanin synthesis can not be ruled out. On the other hand, zinc atoms may be acting as a link between melanin and the protein component in a manner similar to that suggested for iron in the iron pigment of red hair (Flesch, 1968).

Hyperpigmented skin lesions have been reported in patients with abnormalities of trace element metabolism, such as Wilson's disease (Leu *et al.*, 1970) and haemochromatosis (Althausen *et al.*, 1951). Although high serum copper levels are found in a number of hyperpigmentary conditions, like xeroderma pigmentosum (El-Hefnawi *et al.*, 1962) and melanosis accompanying pregnancy or the intake of oral contraceptives, many other conditions with hypercupraemia are not associated with pigmentary changes. In a recent study of vitiligo (Molokhia & Portnoy, 1973), there was no evidence of copper deficiency in either skin or serum, while serum zinc levels were significantly reduced. However, any speculation on the role of trace elements in vitiligo would have to take into account the structural defect which underlies the absence of melanin.

HEAVY METALS

In a strict sense, this group should embrace all metals having a specific gravity more than 5. This would include essential elements like iron, copper and zinc. However, from a biological point of view, the term 'heavy' has long been used in reference to those non-essential and toxic metals and, therefore, no attempt will be made here to change that trend.

Heavy metals can produce skin discolouration in a number of ways. File workers may exhibit black marks in the skin over the face and hands caused by embedded metallic projectiles. A condition named 'gold spinner's hand' has been described by White (1971) in people employed in the manufacture of gold braid for military and other uniforms. Deliberate introduction of heavy metals into the skin takes place in tattooing, where colourful salts of metals like cadmium, chromium, cobalt or mercury are often used. Apart from their original colour, these compounds usually do not elicit any pigmentary reaction in skin.

Melanosis has long been associated with the excessive use of medicaments containing heavy metals like arsenic, bismuth, gold or silver. In these cases, there is an increase in melanin within the basal cell layer and in dermal melanophages. This is thought to be caused by the binding of these heavy metals to tissue sulphydryl groups, thus removing the inhibitory effect which the latter have on tyrosinase activity (Lorincz, 1954). Exposure to ultraviolet light seems to enhance metallic hyperpigmentation, possibly through reducing the compound to its metal, as witnessed by the limitation of dark areas to the exposed skin.

Hyperpigmentation resulting from the use of mercury in cosmetic creams is probably more common than assumed (Burge & Winkelmann, 1970). These products, often used over a long period, cause initial bleaching of skin through the inhibition of melanin formation. This is thought to be achieved by the replacement of copper in tyrosinase with mercury and the subsequent inactivation of that enzyme (Lerner, 1952). However, an opposite effect may result from prolonged use, which can be explained, at least in part, by the binding action of mercury on sulphydryl groups.

The particles of the metal itself may contribute to the colour of skin and can often be demonstrated directly in sections (Burge & Winkelmann, 1970). However, in the absence of a proper history of intake, the identification of the metal may present difficulties and it may be necessary to employ ultrasensitive techniques like neutron activation analysis (Schropl, Oehlschlaegel & Drabner, 1968).

The use of EDTA in skin complications following gold therapy has been advocated by Bureau (1967), in view of the renewed interest shown by rheumatologists in prescribing this heavy metal. Our findings (unpublished data) suggest that gold can be retained by skin for a considerable length of time following its administration, and such an approach may, therefore, be justified in patients with a diminished ability to excrete gold.

MOHAMED M. MOLOKHIA
BENJAMIN PORTNOY

Manchester and Salford Hospital for
Skin Diseases, Quay Street,
Manchester M3 3HL

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Obituaries

A.M.MEMMESHEIMER

Emeritus Professor A.M.Memmesheimer, an Honorary Foreign Member of the British Association of Dermatology (1959), died suddenly at his home in Essen on 14 January 1973.

Alois Matthias Memmesheimer was born in Saarbrücken in 1894, studied at the Universities of Berlin and Bonn, and graduated in Medicine in 1920. He was Assistant successively in Düsseldorf, Essen and Bonn, and in 1925–26 in Cincinnati, Ohio. In 1926 he became Privatdozent in Bonn, and in 1930 was appointed Professor of Dermatology in Tübingen. In 1931 he became an honorary member of the Dermatological Society of London and a member of the Royal Society of Medicine. In November 1931 he was transferred to Essen, where he spent the rest of his career. In 1933 he was put under police surveillance for his outspoken criticism of Nazism, and in 1934 actually imprisoned for the same cause and suspended from his duties for a spell, but in 1939 he put his country first, and served in the German Navy as an Adviser in Dermatology. He had served in the Navy in the First World War, as a combatant. His principal original researches were on epidermophytosis in miners, and he played a prominent part in the German Dermatological Society, being its President from 1960 to 1965. He travelled widely, visiting the U.S.A. frequently, Japan, and most European countries, often as official representative of the GDS, a rôle for which his fluency in several languages and his natural bonhomie fitted him admirably. He was an Honorary Member of the Austrian, Hungarian and American Dermatological Societies, as well as of the British Association of Dermatology.

J.FERGUSON-SMITH

PETER POPCHRISTOV

Professor Dr Peter Popchristov, the outstanding Bulgarian dermatologist and brilliant ambassador of Bulgarian dermatology abroad, died on 10 February 1973, aged 69. Among many international distinctions, he was an Honorary Foreign Member of the British Association of Dermatologists.

His contributions to dermatology are immense and they reflect his many-sided interests and his

inexhaustible energy. He is the author of some 250 papers and of several monographs, among which are the impressive 'Skin Diseases in Children', 'Diseases of the Buccal Mucosa' and 'Dermatological Therapy'—all translated into Russian. Shortly before his death, appeared the work that crowned his career (in collaboration with a few other Bulgarian dermatologists); the 'Textbook of Dermatology', which with its concise form, well proportioned chapters, modern vision of dermatological problems, and up-to-date information may be listed among the best books of its kind.

I had the pleasure to accompany Professor Popchristov on his first trip to Britain, at the invitation of Darrell Wilkinson. He enjoyed this trip immensely. Dazed by the drive of Darrell's programme, he was none the less his match in vivacity and restlessness. He was fascinated and charmed to find out that the British are not cold, stiff, formal, and distant (as an age of literary tradition has represented them), but on the contrary a very hospitable and friendly people. He possessed the same traits, which made him such a kind and lovable person.

His private life reflects his charming personality. He was surrounded by devoted friends, and I had the privilege to be one of them. He enjoyed life in the same manner as he enjoyed his work, with all his heart and passion. There are many British colleagues who have recorded his non-professional, but moving, performances of Bulgarian national songs.

His sudden death was an unexpected blow to his family, to his many friends, and to dermatology. He will be missed for many years to come.

Fiat tibi terra levis, professore!

V.C.ANDREEV

Society proceedings

Scottish Dermatological Society

President: DR W.FRAIN-BELL

A meeting of the Scottish Dermatological Society was held at the Dundee Royal Infirmary on 8 February 1973. In addition to the clinical section, the following papers were presented:

HL-A Antigens in Dermatitis Herpetiformis

DR A.G.WHITE, DR R.ST C.BARNETSON, DR J.A.DA COSTA AND DR D.B.L.MCCLELLAND

Experience with a Suction Blister Device

DR D.F.BARRETT

Effect of Light in Discoid Lupus Erythematosus

DR T.L.PATHI

In vitro Tests of Lymphocyte Function in Chronic Polymorphic Light Eruptions

DR E.J.RAFFLE

Correspondence

THE EFFECTS OF LOCAL CORTICOSTEROID WITHDRAWAL ON THE KOEBNER REACTION IN THE INACTIVE PSORIATIC PATIENT

SIR, At present there is no simple test for detecting patients with the psoriatic diathesis; the vascular (Holti, 1964) and neurological (Weddell *et al.*, 1965) changes described in latent subjects have not been confirmed. Damage to unaffected skin is known to induce psoriasis in a proportion of patients with active psoriasis—the Koebner phenomenon (Eddy, Ascheim & Farber, 1964).

I would like to report the findings of a study designed to induce psoriasis in patients with inactive disease. There is some evidence to suggest that steroid withdrawal may promote psoriasis. Psoriatic lesions may recur more quickly after topical steroid therapy than after treatment with other topical agents (Frank *et al.*, 1964; Rook, 1966; Farber & McLintock, 1968) although this has also been refuted (Shuster & Comaish 1966; Suurmond, 1967). In addition, whilst restricted therapy with topical steroids under occlusion may be followed by recurrence of psoriasis at healed sites (Frank *et al.*, 1964), after systemic therapy lesions may appear at previously unaffected sites (Church, 1965).

In this study trauma was combined with a local steroid withdrawal effect. Methylprednisolone acetate was injected at a site in the skin, and when its effect was judged to be waning the site was damaged.

Thirty patients with inactive psoriasis took part in the investigation. All investigations were carried out on the front of the forearm. The test site was demarcated by scratching a circle of about 1 cm diameter with a needle and 0·2 ml methylprednisolone acetate (40 mg/ml) (Depo-Medrone, Upjohn) was injected low intradermally into the site. The patients were divided into three groups of ten and the injected sites were scraped to bleeding with a scalpel blade at 1, 2, and 3 weeks respectively. The sites were inspected at intervals of 2 weeks for 2 months. Psoriasis did not develop at any of the test sites.

It is concluded that the combination of steroid withdrawal and skin damage did not induce the Koebner reaction in patients with inactive psoriasis and is unlikely to be of value in detecting patients with latent psoriasis.

L. STANKLER

University of Aberdeen

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GRANULOMATOUS *TRICHOPHYTON RUBRUM* INFECTION OF THE PINNAE

SIR, The rarity of fungal granulomata on the pinnae prompts me to report the following case.

Mr W.B., aged 51, was seen in November 1972 at the request of an ENT surgeon, with what were described as peculiar irritant swellings of both auricles. The swellings had been present for over 2 years and were increasing in size. Since 1946 the patient had had an irritable eruption over the distal half of the limbs and scaling in all the toe spaces. The patient was a cardiac cripple with coarctation of the aorta and mitral incompetence. He was receiving a variety of drugs for congestive cardiac failure, and also warfarin, the latter following a cerebral embolism in 1971.

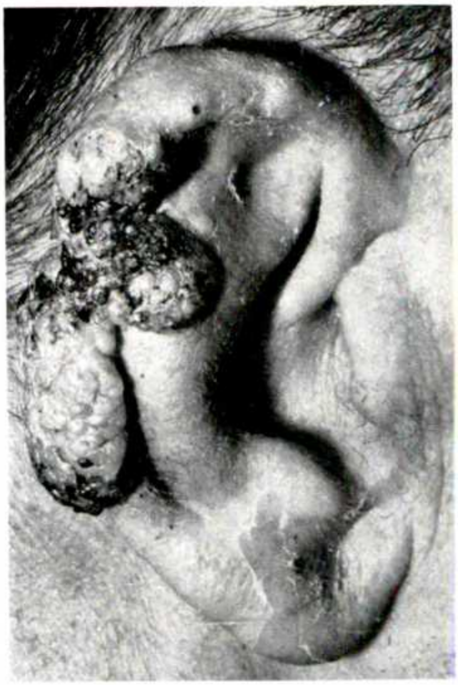

FIGURE 1. Right pinna showing fungal granulomata—pre-treatment.

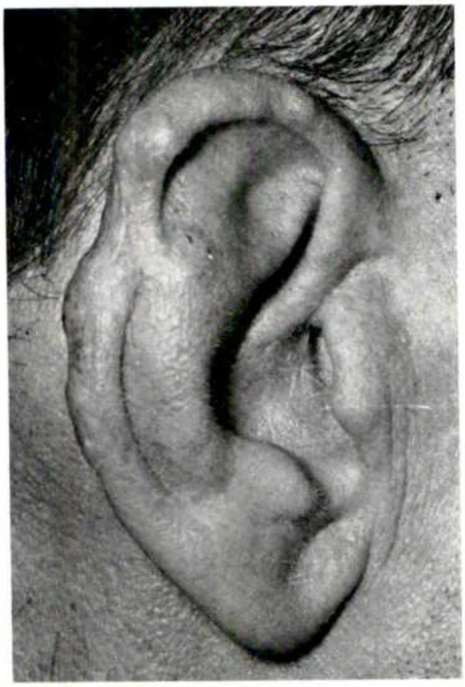

FIGURE 2. Right pinna after 4½ months griseofulvin therapy.

On skin examination, he showed a confluent macular, erythemato-squamous eruption with a well-defined proximal margin in a glove and sock distribution, but sparing palms and soles. Toe and finger nails were markedly thickened. Granulomatous lesions, some fungating, were present on both pinnae, but particularly on the right (Fig. 1). A scraping from the leg for fungal mycelium was positive and material was sent for culture. A biopsy was taken from a granulomatous area on the right pinna. *Trichophyton rubrum* grew from the leg skin scrapings. The histopathology of the ear lesion (Dr T.W.Stewart) revealed a granulomatous infiltrate containing numerous eosinophils and fungal spores. A PAS stain showed hyphae and spores in the keratin and the dermis.

Treatment with oral griseofulvin, 500 mg daily, was initiated and the dose was increased to 1 g daily in early December. Within 2 weeks of treatment the ear lesions were smaller and improvement of all affected areas has been progressive. He has now received griseofulvin for nearly 5 months (Fig. 2) and will probably have a 12-month course.

JULIAN VERBOV

Department of Dermatology
Broadgreen Hospital
Thomas Drive
Liverpool L14 3LB

A VISIT TO THE SOVIET UNION

Sir, A recent visit to Moscow to take part in a symposium on topical steroids afforded us the opportunity of a brief glimpse of Soviet dermatology. The meeting was held at the Central Dermatology and Venereology Scientific Institute (Director: Professor Turanov) and we were able to visit the First Moscow Dermatology Clinic through the kindness of its director, Professor Babayants.

The organization of dermatology in the Soviet Union is essentially as described by Kerdel-Vegas (1962), although the number of dermatologists throughout the country may now be about 12,000.

The First Moscow Clinic, which celebrated its centenary in 1969, is similar in structure to many of the large dermato-venereological clinics of central Europe, which were established during the nineteenth century as independent units with a large number of beds. Use is still made of some therapeutic procedures, such as balneotherapy and hydrotherapy, which have become obsolete here. However, some therapeutic innovations were also to be seen, such as the use of a laser beam for chronic ulcers and a 2-week starvation regime for certain dermatoses, including psoriasis and eczema. Parenteral penicillin was used for systemic sclerosis; rosacea was being treated by the topical application of chloromycetin in 25% dimethyl sulphoxide and dimethyl sulphoxide was also used locally for morphoea.

Numerous seminar rooms are available for groups of students, equipped with various types of teaching aids including an automated self-examination machine which had been developed by Professor Babayants. A large collection of moulages housed in a museum was used extensively.

The medical staff are entirely familiar with Western European and American dermatological literature, which is available in a central library, though not in individual clinics. Most types of therapy including antibiotics, steroids and immunosuppressive drugs are used, though often on a limited scale and in special centres.

Although some interchange visits have already been made with dermatologists from the Soviet Union and Western Countries, these have mainly been undertaken by senior specialists and were necessarily brief. It appeared to us that more knowledge could be exchanged with mutual benefit by visits of staff at a more junior level and limited to one centre at a time—one might suggest at the level of senior registrar or young consultant.

We should particularly like to record our appreciation of the courtesy, kindness and hospitality shown to us by members of the staff at the departments in Moscow who contributed so much to the enjoyment and success of our visit.

Institute of Dermatology, Lisle St. London, W.C.2,
Manchester and Salford Hospital for Skin Diseases,
Quay Street, Manchester M3 3HL and
I.C.I. Pharmaceutical Division, Alderley Park, Macclesfield

C.D.Calnan
Benjamin Portnoy
C.W.Marsden

REFERENCE

Kerdel-Vegas, F. (1962) Dermatology in the Soviet Union. *Transactions and Annual Report of the St John's Hospital Dermatological Society*, **48**, 178.

Book Reviews

Handbuch der Medizinischen Radiologie, Encyclopedia of Medical Radiology, Vol. 19, Part 1. **Spezielle Strahlentherapie maligner Tumoren, Radiation Therapy of Malignant Tumours.** A.Zuppinger and E.Krokowski (Eds.) (1972) Berlin, Heidelberg, New York: Springer. Pp. 824 (77 in English), 270 illustrations. Price £37.32.

This authoritative and well-produced volume is a tribute both to the practical experience and scholarship of a group of contributors from West Germany, Great Britain, the Netherlands, Sweden and Switzerland, and to the able editorship of Professor A.Zuppinger of Bern and Professor E.Krokowski of Kassel. Nearly two-thirds of the text is of immediate interest to dermatologists as a whole throughout the world. Most of the text is in German; there are two chapters (77 pages) in English on 'Oral Cavity Cancer' by Dr R.Gibb and Dr I.D.H.Todd, and on 'Cancer of the Lips' by Dr R.C.S.Pointon, both contributions from the Christie Hospital and Holt Radium Institute, Manchester. A useful feature is the separate Subject Index in English with the German equivalents, in addition to the German Subject Index, which includes English equivalents.

The general approach and philosophy of the Editors reflect the modern conception of clinical oncology and may be illustrated by the following quotations from the English version of the Preface. 'Radiation therapy of malignant tumours has been undergoing extensive evolution for more than two decades now. This is mainly the result of the emergence of supervoltage therapy, not fully exhausted. The second major factor is the acquisition of new knowledge concerning radiation and tumour biology. . . . Today, except in emergency situations, the proper treatment is discussed by a team of surgeons, radiologists, and frequently chemotherapists. . . . Other topics, such as the use of hormonal or chemotherapy, as well as the results of research in tumour biology, are also discussed in this volume'. In addition, it must be mentioned that immunological factors are discussed, particularly in relation to carcinogenesis and to the problems of malignant melanoma and its treatment.

Chapter A by Professor G.J.van der Plaats, of Maastricht, deals with precanceroses of the skin and mucous membranes, in gynaecology and in internal organs, mainly lungs and gastro-intestinal tract. The importance of treatment in the precancerous stages is emphasized in a very practical way.

Chapter B on 'Skin' is a monograph of 240 pages, including 96 pages on malignant melanoma, by Professor H.Storck and his colleagues Dr K.Schwarz and Dr F.Ott, of the University Dermatological Clinic of the Cantonal Hospital, Zürich. This is in the tradition developing from the work of G.Miescher, and is remarkably comprehensive from both the fundamental and practical aspects. The experience with the increasing use of high energy electrons in therapy is important, and the section on mycosis fungoides is particularly interesting.

There is a great deal of information about malignant tumours of the eye, including eyelids, in Chapter C by Professor K.Hohl of St Gallen. Cancer of the nasal cavity and accessory nasal sinuses is considered in an authoritative chapter by Professor H.Oeser and Dr W.Schlungbaum, of West Berlin. A clear account of the treatment of cancer of the oral cavity is given by Dr R.Gibb and Dr I.D.H.Todd of Manchester. Again, the experience of the Christie Hospital and Holt Radium Institute, Manchester, is described in the chapter by Dr R.C.S.Pointon on 'Cancer of the Lips'. Complementary to this is the following chapter by Professor W.Hellriegel of Stuttgart on the percutaneous irradiation of tumours of the lips. Of particular importance is his conclusion that at the present time, high energy electron therapy of cancer of the lip (with 5–8 MeV electrons) is the method of choice.

The rest of the volume, about one-third of the text, consists of seven equally authoritative chapters on malignant disease of the gastro-intestinal tract. However, this work will not be considered in detail here because it is not primarily of interest to dermatologists.

Full references to the literature, including some for the year 1971, are given with each chapter. The illustrations and figures are almost invariably well chosen and informative. The production as a whole is of an extremely high standard.

This is a very good book and an important one for dermatologists. It should be a work of reference in the libraries of all Departments of Dermatology, though unfortunately its price will limit its purchase by private individuals.

JOSEPH S.MITCHELL

Department of Radiotherapeutics
University of Cambridge

Lecture Notes on Dermatology. BETHEL SOLOMONS (1973) 3rd edition. Oxford: Blackwell Scientific Publications. Pp. ix+265, 70 black and white illustrations, 37 colour plates. Price £2.25.

The third edition of this small book covering the common conditions seen in dermatology follows 4 years after the second edition. The preface describes it as being intended to be an introduction to dermatology for students and general practitioners. It is an indictment of our medical school curricula if a doctor is not introduced to dermatology until he becomes a general practitioner. Changes in the present edition refer chiefly to treatment with immunosuppressive drugs, such as methotrexate and 5-fluorouracil, trisoralens, and also to the presence of *Cheyletiella* in the U.K., but it is a pity that none of these items has reached the index. The common dermatoses are described interestingly, and simple practical treatment is recommended.

The limitations of writing such a compressed account of the subject are so obvious that it seems churlish to offer criticisms of detail, but it is disappointing to see the quality of the colour photography uneven, so as to be misleading in some conditions, and one photograph out of focus (xanthomata). Some recommendations for treatment also need to be up-dated, for example, X-ray treatment for paronychia, steroid injections for alopecia areata, and also the use of phenol on a toothbrush for the same condition. Many dermatologists would not consider cautery or curetting the best treatment for plantar warts, nor strong erythema doses of ultraviolet light a proven preventative of chilblains, nor is it true that urticaria is common in dermographism. The term candidiasis replaced moniliasis some years ago.

R.R.M.HARMAN

Dermatologie. Ein Repertorium. 2. Auflage. KURT WINKLER (1973) Berlin, New York: De Gruyter. Pp. vii+281, 128 illustrations in black and white, 75 in colour. Price £11.04.

Inevitably this pocket size, rapid reference book intended for the senior student, junior non-dermatological hospital staff, and the general practitioner on his rounds has grown since its first edition in 1968 from 168 to 249 pages. Most of the book is taken up by brief, yet often amazingly precise and vivid descriptions of the characteristic clinical features of common as well as of many rare disorders, their differential diagnoses and management. The very short second part of the book lists merely regional afflictions as a diagnostic aid and signposts for the baffled clinician, but may also help those concerned with multiple choice questionnaires.

This edition also includes some dermatoses which are now seen in Germany due to increased tourism and the presence of foreign workers from Eastern Mediterranean countries. However, there is also much additional and improved information on management. Nevertheless, there is still room for weeding out therapeutic suggestions for which there is neither rational nor empirical justification, such as the avoidance of nylon shirts for those afflicted with tinea versicolor, or which are therapeutically worthless, yet potentially toxic, measures, such as Calciferol for chilblains or the use of topical vasodilators.

A major shortcoming of this book is its almost complete lack of emphasis on investigations, either for diagnostic purposes or to assess activity of the disease process, or as a guide to rational treatment. In spite of much 'up-dating' there are quite notable omissions, such as the failure to relate hereditary angioneurotic oedema to the lack of the inhibitor of the activated first component of complement and to mention the life saving treatment with fresh plasma, while epsilon-amino-caproic acid, the prophylactic treatment for this condition, is mentioned next to antihistamines in the long list of potentially useful treatments in urticaria. The seemingly uncritical acceptance of customary, but now increasingly challenged, authoritarian statements is perhaps inevitable in such a short text, which nevertheless allowed room for the statement that psoriasis may be related to a disturbance of the dehydroepiandrosterone metabolism and mentions its controversial myopathy with the same emphasis as its arthropathy.

Inspite of its shortcomings this edition is a great improvement on its predecessor, and among pocket reference and revision books it must be one of the best available.

The publishers must be congratulated on the very high standard of production, in particular of the

128 black and white and 75 colour pictures, every one being instantly diagnosable. This little book will no doubt enjoy increasing popularity and with further improvements, in particular rigorous pruning of outdated material, further up-dating of pathological data, and the inclusion of many more key references, it will become highly recommendable.

G.HOLTI

Pathogenic Mycoplasmas. A CIBA FOUNDATION SYMPOSIUM (1972) Amsterdam: Associated Scientific Publishers. Pp. 404, 97 illustrations. Price £7.25.

Mycoplasmas are being associated with an increasing number of human, animal and plant diseases, but in many of these the aetiological side of the mycoplasma remains to be verified. In this Symposium on Pathogenic Mycoplasmas, held at the Ciba Foundation, London, in January 1972, many participants from all over the world shared their experiences and their problems in this expanding field.

This symposium starts with an introduction on the principles of 'Mini-Life', where a brief historical background is given and where the place of mycoplasmas within the microbiology field is defined, particularly in relation to bacteria and viruses.

Following this, there is a discussion of the criteria of pathogenicity of mycoplasmas, the presence of virulence factors in certain species, and the epidemiology of the contamination of cell cultures by these organisms.

Several papers deal with the cytology and immunochemistry of mycoplasmas, their growth requirements, their isolation, and certain identification methods, such as plate epi-immunofluorescence for their rapid detection; this method is based on staining colonies of mycoplasmas in agar cultures with specific conjugated mycoplasma antisera.

Other papers deal with more specific subjects, such as mycoplasmas affecting citrus plants, and others associated with the corn stunt disease. One paper deals with the possibility of mycoplasmas as being the cause of sterility in some male hybrids of *Drosophila paulistorum.* A later paper discusses the possible pathogenicity of mycoplasmas in rheumatoid arthritis in man, where *Mycoplasma fermentans* was isolated from 39% of rheumatoid synovial effusions. The pathogenicity of *M. pneumoniae,* the only proven human pathogen so far, is presented in another paper, as studied in the human foetal trachea in organ culture—mainly the effect on ciliary activity of the respiratory epithelium by light, immunofluorescence and electron microscopy. Few other papers deal with the interaction between mycoplasmas and respiratory viruses as well as between mycoplasmas and malarial parasites. Here the emphasis is on the enhancement of mycoplasmal growth in tissues that are already damaged or stressed by a previous or concomitant viral or parasitic infection.

A final paper deals with cell-mediated immunity and the response to mycoplasma infections. The fact that humoral circulating antibody does not protect fully against disease caused by mycoplasmas suggests that cell-mediated immunity might play some role in these infections. The way in which these cell-mediated immunity mechanisms operate, the relation of this type of immunity to other mycoplasma infections, and the possible role it may have in mycoplasmas host specificity are discussed.

There are brief references to the association of mycoplasmas with septic abortion and non-specific urethritis, the latter concerning the T-mycoplasmas in particular. For the dermatologist, there is no mention in this symposium of the few dermatological entities that are suspected sometimes to be associated with mycoplasmas.

The main emphasis in this book, which is particularly reflected through the discussions following the various papers, is on the problems and techniques of isolation, identification and culture, as well as on the usefulness of immunological approaches in this field.

This book also offers a good selection of references. It is not intended to be a basic textbook on mycoplasmas. It mainly is intended to present the various experiences and recent findings of leading workers in the field of mycoplasma research and the problems they face. It also offers many suggestions for the future.

MUNA MATTA

British Journal of Dermatology (1973) **89**, 219.

Quarterly Review

CONTACT DERMATITIS XXIII

The Mafenide Story

The use of topical antimicrobial therapy, primarily mafenide hydrochloride and mafenide acetate, has significantly lowered the mortality from burn wound sepsis (Dressler *et al.*, 1967; Yaffee & Dressler, 1969). Rittenbury *et al.* (1966) could show experimentally that bacterial invasion of the burn occurs at a predictable rate and that prognosis is related to numbers of bacteria in a wound. Therefore, the prophylactic topical application of mafenide acetate or mafenide hydrochloride in the first hours following a large burn reduces mortality significantly (Dressler *et al.*, 1967). Because the use of mafenide hydrochloride has been associated with hyperchloraemic acidosis, Dressler *et al.* (1967) prefer mafenide acetate to mafenide hydrochloride. Nevertheless, some reports dealing with adverse side-effects (Velasco & Africk, 1971; Yaffee & Dressler, 1969) do not state clearly whether these patients were treated with mafenide acetate alone or with mixtures of the acetate and the hydrochloride.

These new therapeutic recommendations stand in obvious contradiction of the statement that mafenide is one of the few medicaments which should be abandoned because of their allergic potencies (Bandmann & Dohn, 1967).

Chemically, mafenide is 4-amino-methyl-benzene-sulphonamide; because of its action it is numbered among the sulphonamides, although its structure is not strictly that of these drugs (Walter & Heilmayr, 1969). Unlike the sulphonamides, the amino group is not directly in para position on the benzene ring, but is separated from this ring by a methyl group. Therefore, mafenide has an aliphatic and the sulphonamides an aromatic quality. The chemical properties of an aliphatic amino group differ essentially from those of an amino group directly attached to the benzene ring; in particular it is much more strongly basic (Schreus, 1950).

In many countries mafenide is marketed in combination with sulphonamides in a powder for topical application. After the introduction of the Marfanil–Prontalbin powder at the end of the second world war and its extensive use in the hospitals of the German army, an increasing number of contact allergies to this product were reported (Schreus, 1950; Schultz, 1962). The very first studies already revealed the fact that in many cases patch tests were positive only to mafenide and not to sulphonamides (Koch, 1949). In the years 1960–65 mafenide held the second place in the ranking order of drug allergies; with 437 cases in the Munich dermatological department, it was outnumbered only by benzocaine with 765 positives (Bandmann, 1966).

An evaluation of test results for the years 1964–66 shows that 162 patients of a total of 3914 patch tested reacted to mafenide (Bandmann, 1967). Contrary to the assumption of Schulz (1962) and of Bandmann & Dohn (1967) that the number of mafenide sensitivities is decreasing, the authors found, for the years 1970–72, that 242 of 4394 tested eczematous patients reacted to mafenide. It should be mentioned that older patients of course predominated, but, nevertheless, eleven patients were in their third decade. This large number of mafenide contact allergies led to the statement that the standard test series recommended by the ICDRG is not adequate for Germany, particularly because it does not include mafenide (Bandmann *et al.*, 1972).

For patch testing purposes mafenide hydrochloride has to be used at 10% in petrolatum, following the normal Al-test routine procedure. The duration of exposure has to be at least 24 h and readings have to be taken after 48 and 72 h. A shortening of the exposure time to 8 h leads to false negative results (Hildebrandt, 1973). Mafenide acetate must not be used in patch testing. As early as 1950, Schreus showed that patients who were sensitized to mafenide hydrochloride did not react to mafenide acetate. These results were confirmed by the work of Schulz (1962). It is possible that the negative patch test results of Yaffee & Dressler (1969) could be explained by this fact. Their patients were sensitized to mafenide hydrochloride and patch tested with mafenide acetate.

At an early stage it was pointed out that cross-reactions between mafenide and benzocaine and other related esters of para-aminobenzoic acid occur relatively often (extensive literature in the publication of Schulz, 1962). In our recent study mentioned above, 135 of 242 mafenide sensitive patients showed positive reactions to other chemicals of the so-called para-group.

The allergenic potency of mafenide is linked to the intramolecular distribution of charge, i.e. only an alkaline amino group makes the molecule act as an allergen. The statement of Schreus (1950) and Schulz (1962), that reduction of the basicity affects the therapeutic effect of mafenide, has to be confirmed, in the light of the good therapeutic results reported by Dressler *et al.* (1967). The possibility that mafenide acetate is transformed to mafenide hydrochloride during the treatment of large burns requires investigation.

Sulphonamides do not cross-react with mafenide and other aromatic para-amines (Wurst, 1970). Not even the sulphonamide which is combined with mafenide in the same formulation leads to a concomitant allergy. In rating contact sensitizers by means of the maximization test, Kligman (1966) pointed out that sulphonamides are only mild sensitizers. He called this 'a dramatic example of disparity between the verdicts of clinical usage and maximization testing'. It is very possible that the literature has often incriminated sulphonamides when the real offender was mafenide, which has a different chemical structure.

If in treating burns with mafenide acetate the products used also contain mafenide hydrochloride, or if mafenide acetate is altered to mafenide hydrochloride in the scar, a high rate of sensitization can be expected. This may be a calculated risk if the mortality of large burn wounds due to septicaemia can be reduced; but we warn against the uncritical use of mafenide in minor everyday burns.

HANS JÜRGEN BANDMANN
REINHARD BREIT

Dermatologische Klinik und Poliklinik der Universität
D-8000 München 2
Frauenlobstraße 9
West Germany

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British Journal of Dermatology (1973) **89,** 222.

News and Notices

Investigative Group of the British Association of Dermatology

The next meeting of the Group will be held at the University of Liverpool on Friday and Saturday, 11 and 12 January 1974.

Those requiring accommodation, which on the Friday is available in a Student's Hall of Residence, should write to Dr C. F. H. Vickers, Department of Dermatology, Liverpool Royal Infirmary. Members wishing to submit papers should send standard abstracts (details are being pre-circulated) to Dr M. W. Greaves, Department of Dermatology, Royal Victoria Infirmary, Newcastle-upon-Tyne, not later than 15 October 1973. There will also be facilities for scientific demonstrations, and proposals for these should reach Dr Greaves or Dr Vickers by the same date.

European Society for Dermatological Research

The Fourth Meeting of the European Society for Dermatological Research will take place in the Rai-Congrescentrum in Amsterdam on 23–24 April 1974.

Attendance at the scientific sessions will be open to all members, contributors introduced by members, and guests. Non-members who wish to present a paper are kindly requested to send an abstract of the paper to the Secretary. They will then be invited to attend the meeting either as contributors or guests. Forms for abstracts are available on request to the Secretary.

All abstracts should be sent to Michel Prunieras, Secretary, Fondation Ad. de Rothschild, 29 rue Manin, 75019 Paris, before 15 December 1973.

XX Congress of the Polish Association of Dermatology, Katowice, 16–18 May 1974

The main themes of this congress will be—Occupational skin diseases: Allergic skin diseases: Auto-immune skin diseases: Advances in the diagnosis of venereal diseases and in the campaign against them.

Chairman of the Organizing Committee: Professor Józefa Rubisz-Brzezińska. Secretary: Professor Alina Pietrzykowska-Chorążak, Dermatological Clinic, Street Francuska 20/24 40-027 Katowice, Poland.

Psoriasis Research Award for 1973

The Department of Dermatology, Baylor College of Medicine, Houston, Texas, announces the Mr and Mrs J. N. Taub International Memorial Award for Psoriasis Research for 1973.

This annual award of $1000 was established in 1957 by Carol and Henry J. N. Taub in honour of their grandparents, Mr and Mrs J. N. Taub. The award is designed for the individual or group whose investigative work in this field has been the most outstanding. Names of applicants for the award, together with supporting data, should be submitted for consideration by 1 December 1973. Results of the research must have been presented before a dermatological medical society or published in a recognized medical journal.

The award's recipient will be determined by a committee of seven men. If, in the committee's opinion, none of the applications reflects outstanding research, no award will be given for the year.

Address all communications to: John M. Knox, M.D., Professor and Chairman, Department of Dermatology, Baylor College of Medicine, Houston, Texas 77025, U.S.A.

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Juvenal, Satires VII, 51.

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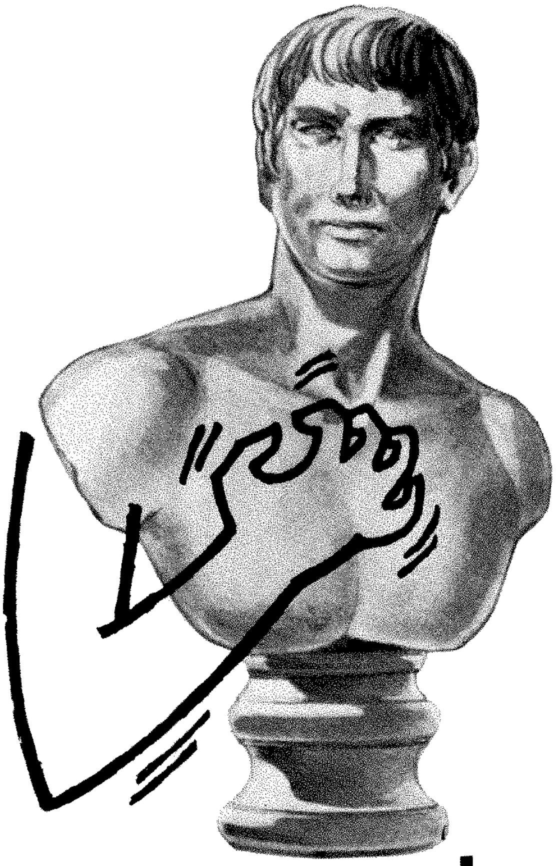

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Journal of the Society of

Cosmetic Chemists

of Great Britain

Edited by J. M. Blakeway

Vol. 24 No. 8 July 1973

C. Prottey, P. J. Hartop and T. F. M. Ferguson. The effect of soap upon certain aspects of skin biochemistry.

M. C. Bidmead and Mitchell N. Rodger. The effect of enzymes on stratum corneum.

R. W. Rance. Studies of the factors controlling the action of hair sprays—1: The spreading of hair spray resin solutions on hair.

K. D. Bingham and D. A. Shaw. Male pattern baldness and the metabolism of androgens by human scalp skin.

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes each month a signed editorial reviewing, primarily for the benefit of the clinician, advances in some field of laboratory research, and also quarterly reviews of recent advances in contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This journal is covered by *Current Contents*.

Manuscripts should be sent to the Editor, Dr A. J. Rook, Old Hall, Steeple Bumpstead, Haverhill, Suffolk. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	percent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations*, A Guide for Biological and Medical Editors and Authors (1971) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs.', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be unmounted glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black Indian ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these legends should be grouped on a separate sheet of paper.

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Page proofs will be submitted to the author for proof-correction (see under 'manuscripts') and should be returned to the Editor within three days. Major alterations from the text cannot be accepted.

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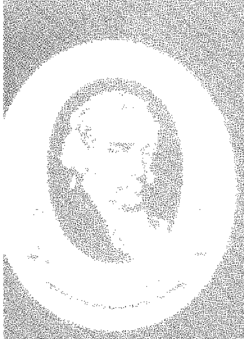

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British Journal of Dermatology

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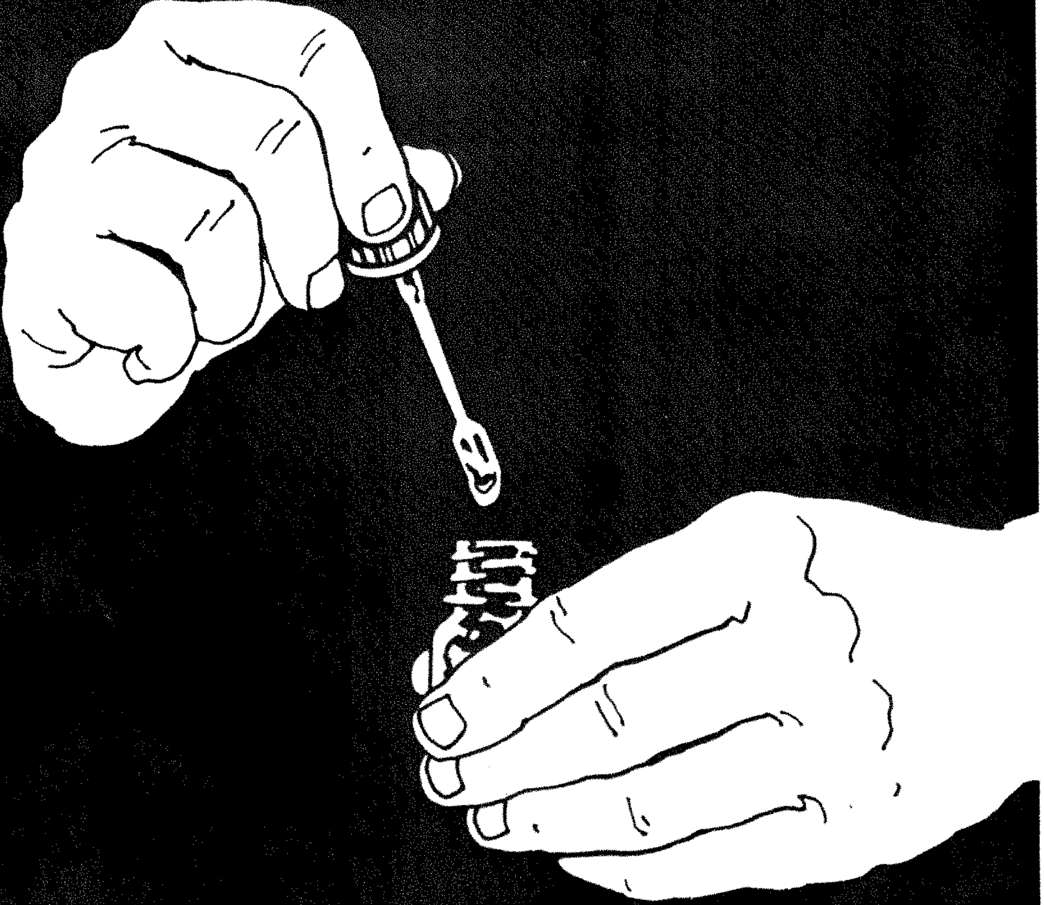

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Refs: Practitioner, 207, 197, 1971.
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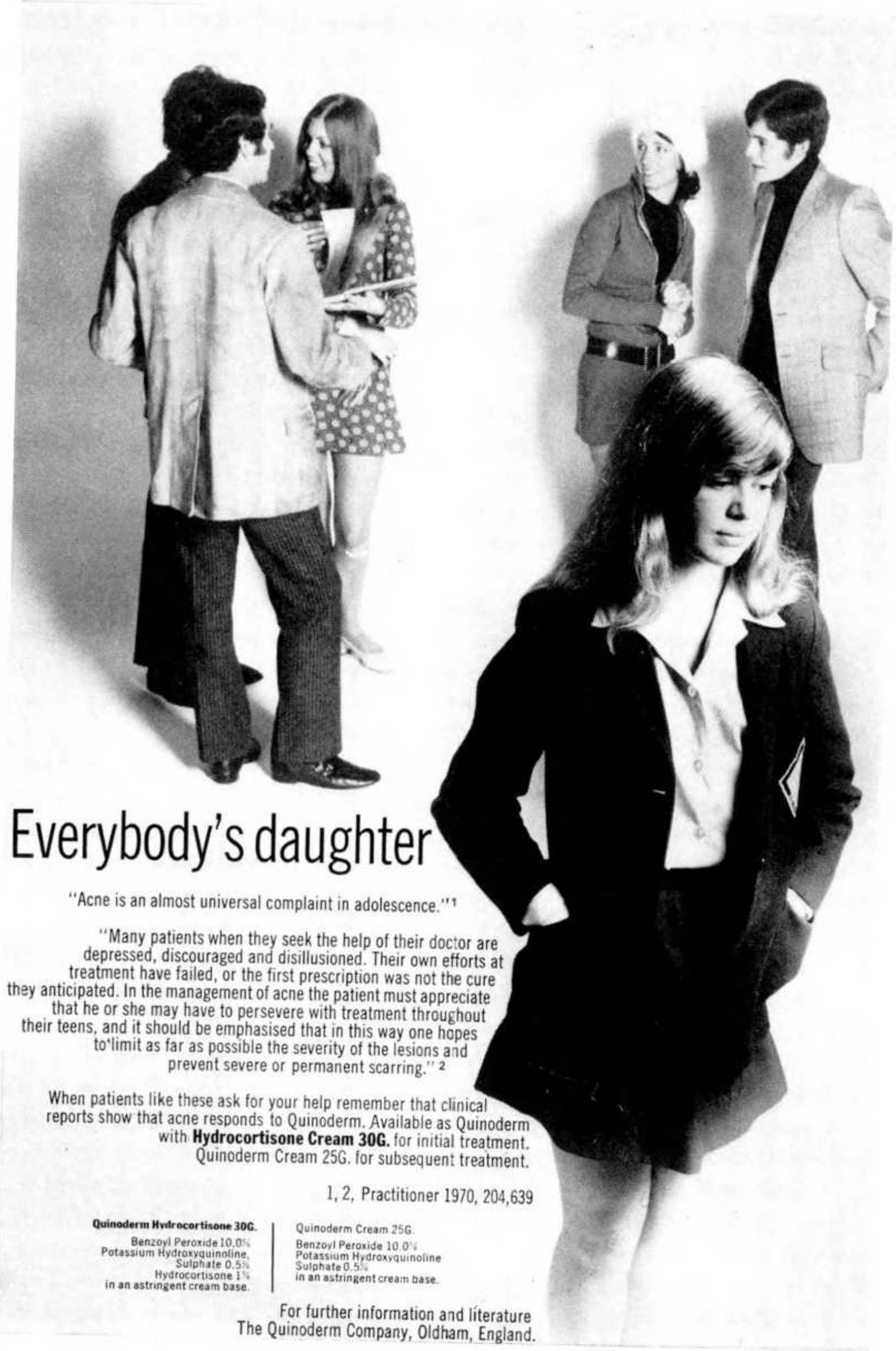

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1, 2, Practitioner 1970, 204,639

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Clin. Trials J. 1970, 4, 438

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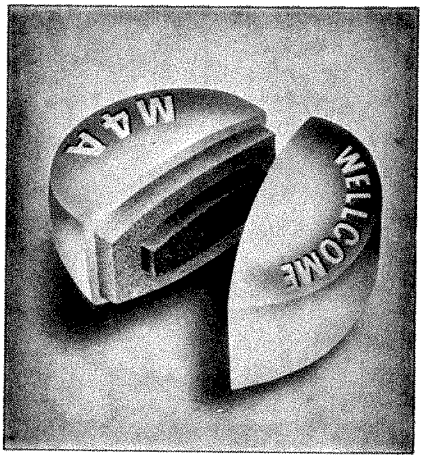
*Trade Mark

†*Eur J clin Pharmacol* (1971), 3, 215.



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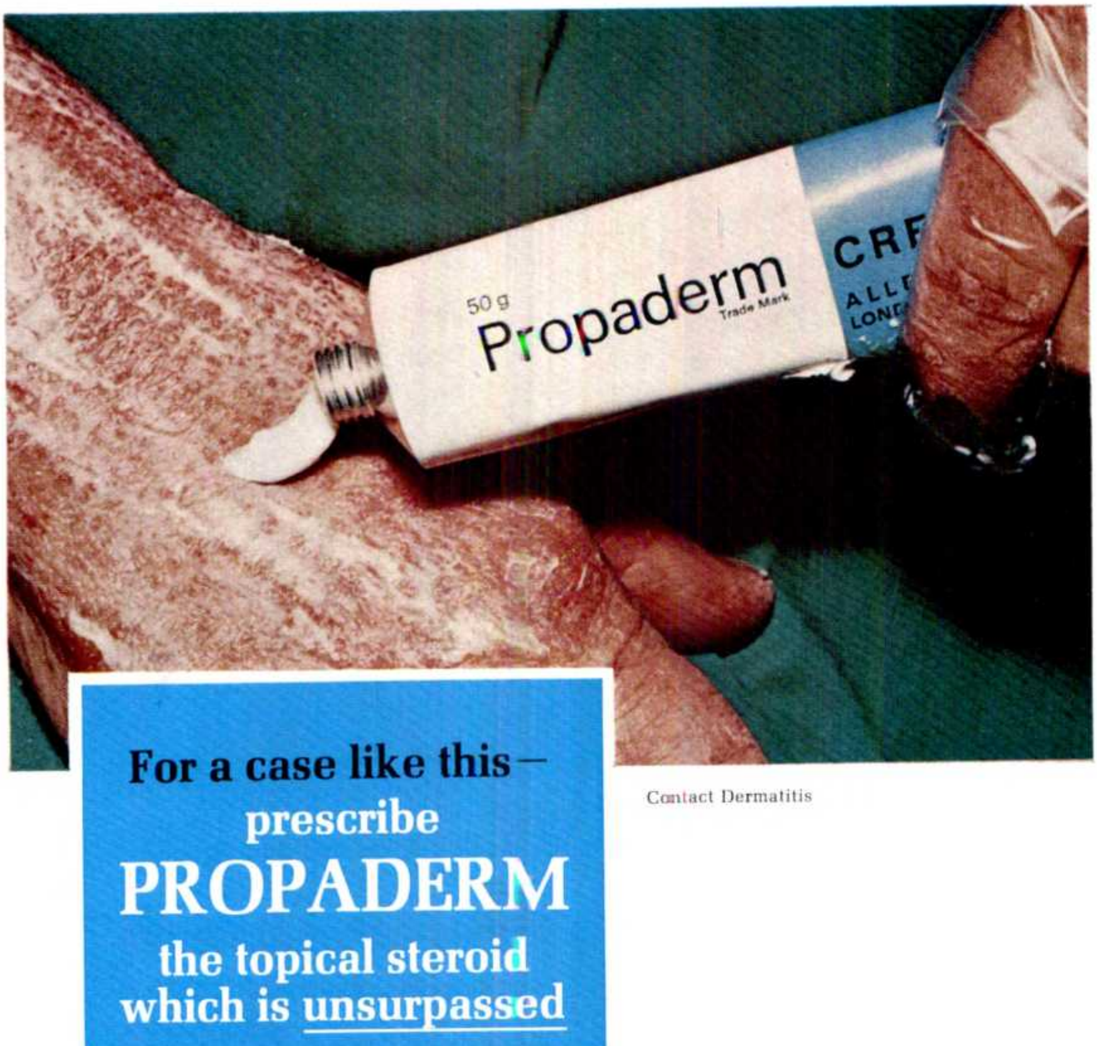

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unsurpassed in clinical effectiveness
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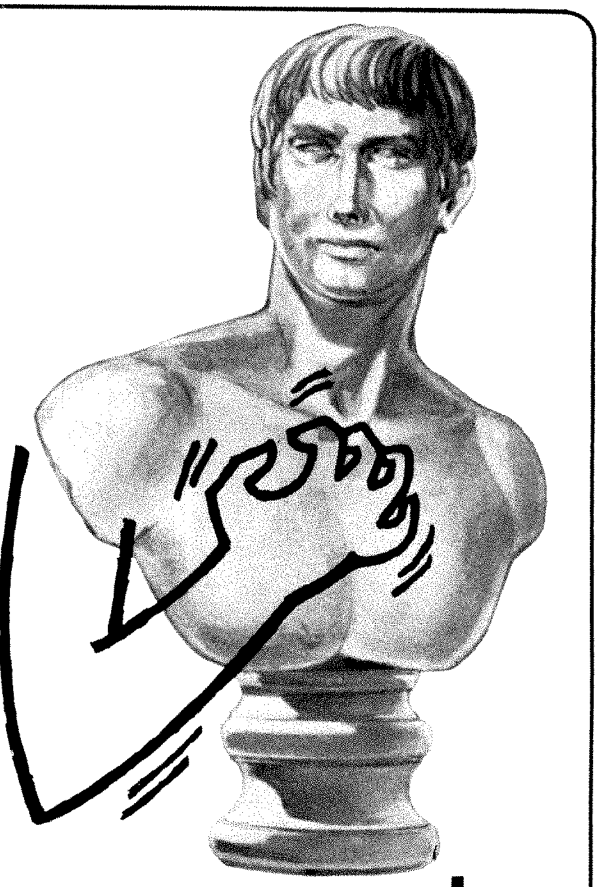

"tenet insanabile multos cacoethes et aegro in corde senescit"

Juvenal, Satires VII, 51.

(many suffer an incurable itch which ages with their ailing body)

But the dermatological itch must be soothed because the patient in scratching only aggravates the condition. In pruritus, a dermatological preparation to be effective should not only be an efficient antipruritic but also a bactericidal and anti-inflammatory agent.

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[1] Data held on file.

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tretinoin

ACNE TREATMENT

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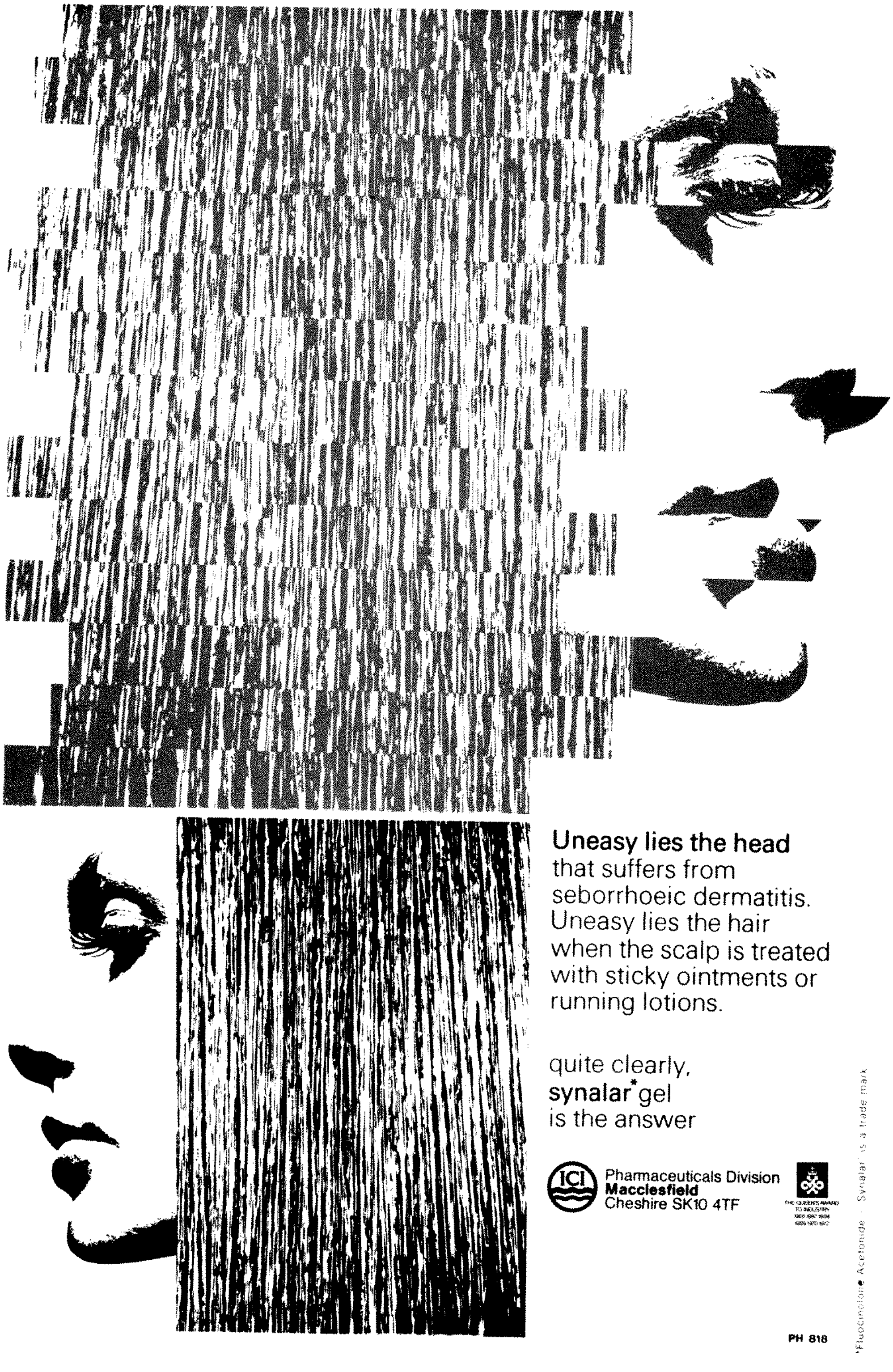

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British Journal of Dermatology (1973) **89**, 223.

Clinical and Laboratory Investigations

Ultrastructural changes in four cases of lichen sclerosus et atrophicus

PATRICIA R. MANN AND M. A. COWAN

MRC Unit on the Experimental Pathology of Skin and United Birmingham Hospitals

Accepted for publication 29 January 1973

SUMMARY

The ultrastructural changes in extragenital lichen sclerosus et atrophicus have been studied in four cases. The most pronounced changes are present in the dermis, where there are collagen degeneration and regeneration and increased amounts of elastin. The basal lamina shows holes, long gaps, and replication. Degenerate dermal material passes into the epidermis, which has increased numbers of Langerhans' cells and decreased numbers of melanocytes. These phenomena are discussed.

Lichen sclerosus et atrophicus (LSA) is an uncommon disorder of the skin and mucous membranes, which has characteristic clinical and histological appearances. Its aetiology is unknown. Electron-microscopic (EM) studies of LSA are few (Forssmann, Holzmann & Cabre, 1964; Nicolau & Balus, 1966). The present EM study was performed on specimens of extragenital LSA obtained from four patients, in order to try to increase the understanding of the aetiology and pathogenesis of the condition. Preliminary findings in two of these patients have been previously reported (Wallace, 1971).

CLINICAL MATERIAL

Case 1

Mrs E.W., aged 62 years, showed multiple, slightly raised, white, infiltrated plaques on the trunk, together with anogenital LSA. Biopsy material was obtained from a typical lesion of the left breast area.

Case 2

Mrs E.D., aged 60 years, had anogenital LSA together with an atrophic, slightly depressed lesion on the inner aspect of the right buttock. The buttock lesion was biopsied.

Case 3

Mrs A.C., aged 69 years. Biopsy was carried out on a shiny, white, infiltrated plaque on the dorsal aspect of the right forearm.

Case 4

Miss B.C., aged 38 years. Biopsy was carried out on a white, linear, slightly depressed lesion on the inner aspect of the right arm, near the elbow.

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No treatment whatsoever had been applied to the lesions of Cases 1 and 2. No topical steroid had been applied to the lesions of Cases 3 and 4 for a period of at least 2 months before biopsy for EM was carried out. In Cases 1 and 3 the lesions were active, i.e. the surface was slightly raised, very white and with palpable infiltration. In Cases 2 and 4, the lesions were inactive or resolving, i.e. the surface was level or slightly depressed, less white than in the active lesions and with little palpable infiltration. All the specimens showed the typical light microscopic features of LSA, namely hyperkeratosis, epidermal atrophy with liquefaction degeneration of basal cells, and a zone of pale homogeneous-looking collagen in the upper dermis with a variable lymphohistiocytic infiltrate below it.

METHODS

Pieces of skin were removed by biopsy under local anaesthesia with 1% plain lignocaine. They were cut into cubes of approximately 1 mm and fixed for 1 h in 2% glutaraldehyde in phosphate buffer pH 7·4 with 0·2 M sucrose added. This was followed by washing in several changes of sucrose–phosphate buffer pH 7·4 over a period of at least 24 h before post-fixation in 2% osmium tetroxide dissolved in the same sucrose–phosphate buffer. Dehydration in graded concentrations of ethanol was followed by embedding in Epon. Sections were cut with a glass knife on a Reichert OMU2 ultramicrotome, and 1 μm survey sections were stained with aqueous toluidine blue. Ultrathin sections were collected on uncoated copper grids and stained with methanolic uranyl acetate.

RESULTS

The EM changes were similar in all the specimens, varying only in degree. The dermal changes were present throughout the depth of the dermis, from immediately below the basal lamina to the lower limit of the section, which was still within the hyaline region seen on light microscopy. Cells and blood vessels were sparse in the regions examined. The most striking abnormalities were in the connective tissue fibres and ground substance. These changes were qualitatively consistent, but varied in degree with the clinical activity of the lesions as defined.

Collagen fibres varied greatly in their diameters (10–90 nm) within the same region and showed gross alterations in their alignment to one another (Figs. 1, 2 and 6). They were frequently associated with deposits of granular material (Fig. 1). Normal cross-striations were present in the thicker fibres, and occasional fibres in cross-section looked like empty tubes (Fig. 2). In active lesions (Cases 1 and 3) larger fibres were more common. In some areas the fibres were quite sparse, and few fine fibrils were present. In resolving lesions (Cases 2 and 4) there was much more evidence of new collagen formation, which was laid down in a random fashion as a network consisting of fine fibrils which rarely showed cross-striations (Fig. 3). Larger, striated collagen fibres were still present, but less common.

Masses of varying size with the EM features of elastin were scattered throughout the dermis, often in close proximity to collagen (Fig. 4). Elastin was seen in larger amounts than in normal skin.

Some dermal fibroblasts contained small clumps of mature collagen still showing cross-striations (Fig. 5). In some cases a lysosomal membrane surrounded the collagen, which had presumably been phagocytosed. There were also macrophages which contained collagen fragments and melanin granules.

Basal lamina changes were consistently present and were similar in all the specimens. A general diffuse thickening of the lamina (up to 500 nm) was seen most frequently. In some areas there were small holes in the lamina, and in others there were zones of complete absence of lamina leaving basal keratinocytes in direct contact with the dermis. In other areas there was marked replication of the

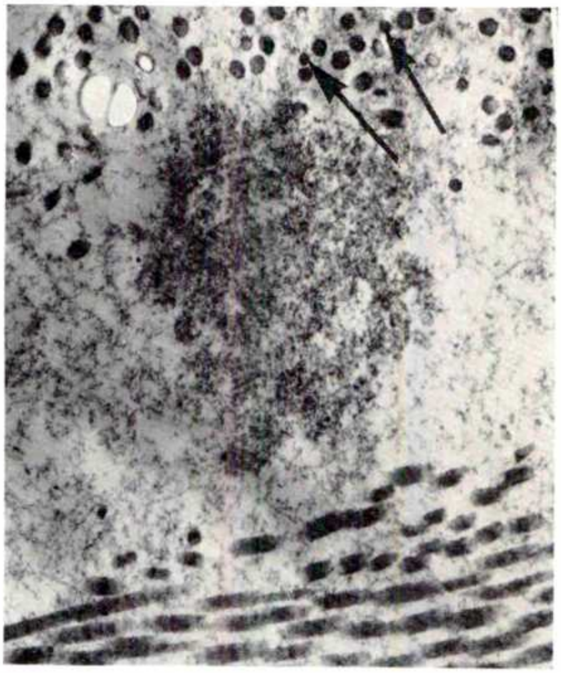

FIGURE 1. Collagen fibres of varying diameters, 40–90 nm (small fibres arrowed), and associated degenerate, granular, dermal material. Clinically active lesion (× 30,000).

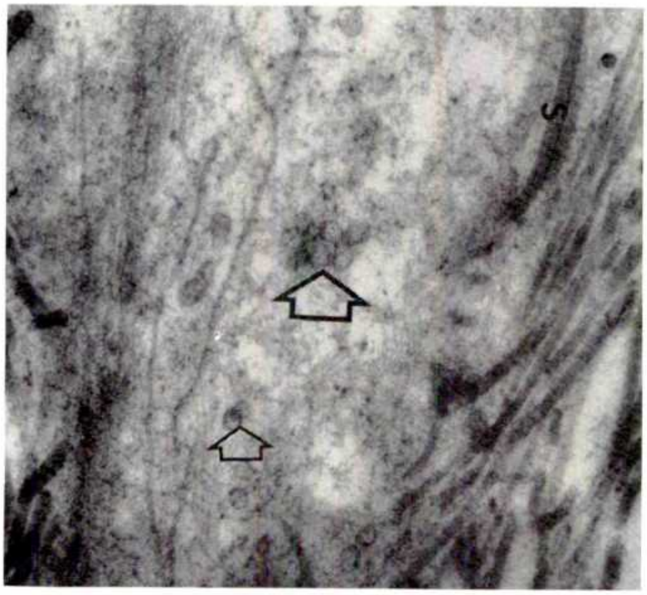

FIGURE 2. Normal collagen fibres with cross-striations (S), and fibres in cross-section appearing as empty tubes (arrow). Diameters vary from 20 to 90 nm (× 31,000).

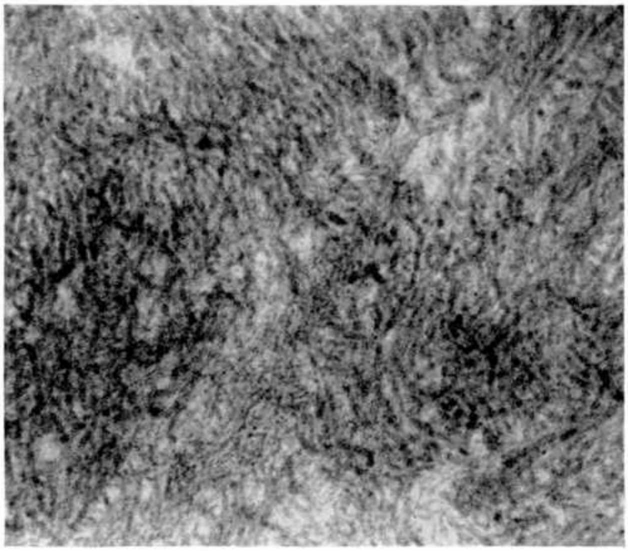

FIGURE 3. Random network of fine collagen fibrils. Diameters vary from 10 to 20 nm. Resolving lesion (× 41,000).

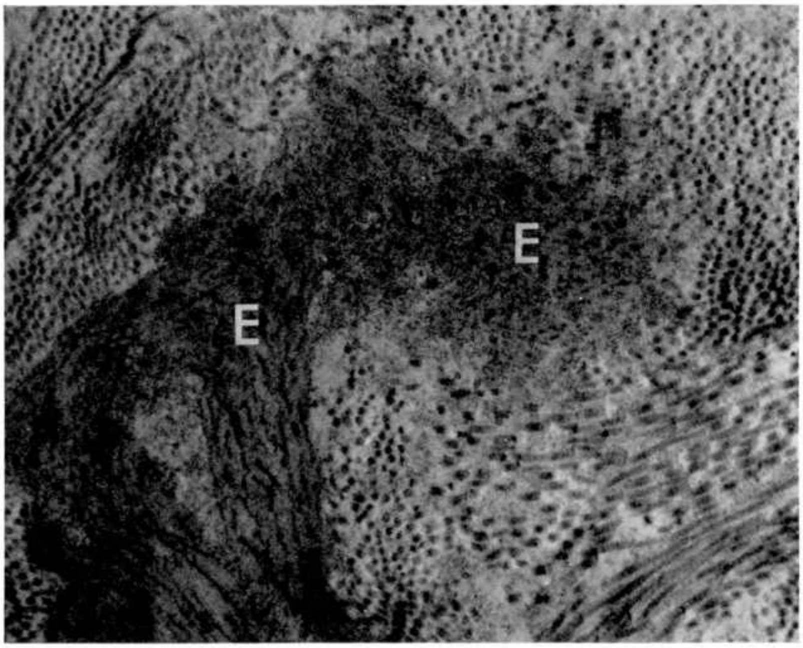

FIGURE 4. Masses of elastin (E) in the dermis (× 13,400).

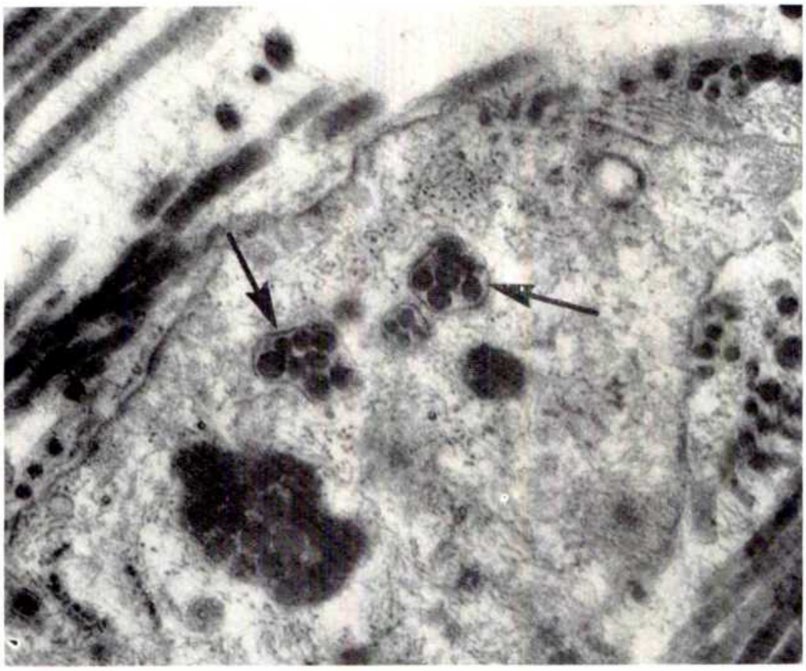

FIGURE 5. Clumps of collagen in cytoplasm of dermal fibroblast. Phagosome membrane arrowed (× 32,000).

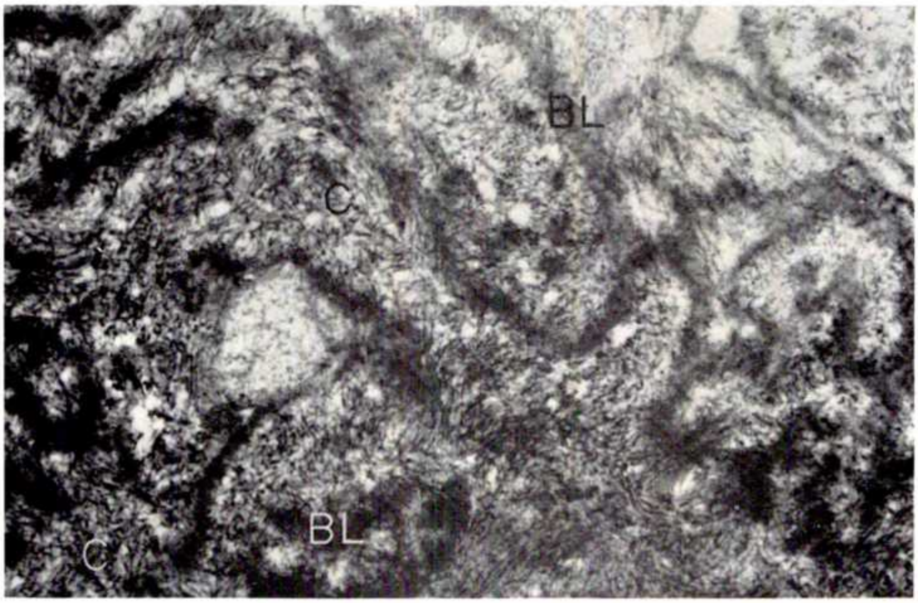

FIGURE 6. Replication of basal lamina (BL). Immature collagen, C (× 6000).

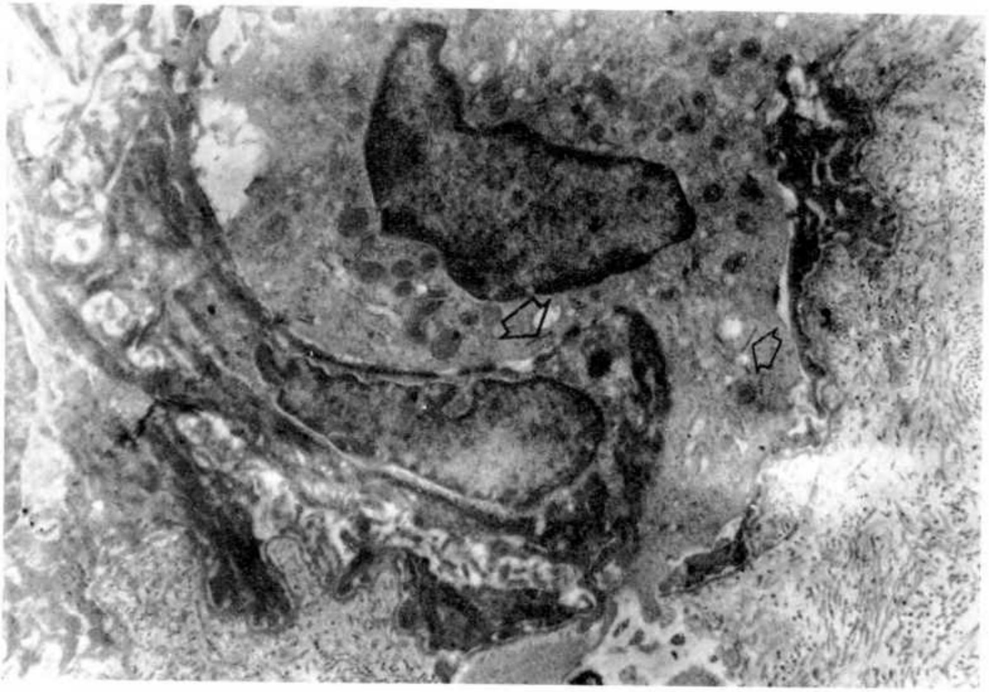

FIGURE 7. Langerhans' cell, part in the epidermis and part in the dermis. Langerhans' granules arrowed (× 7500).

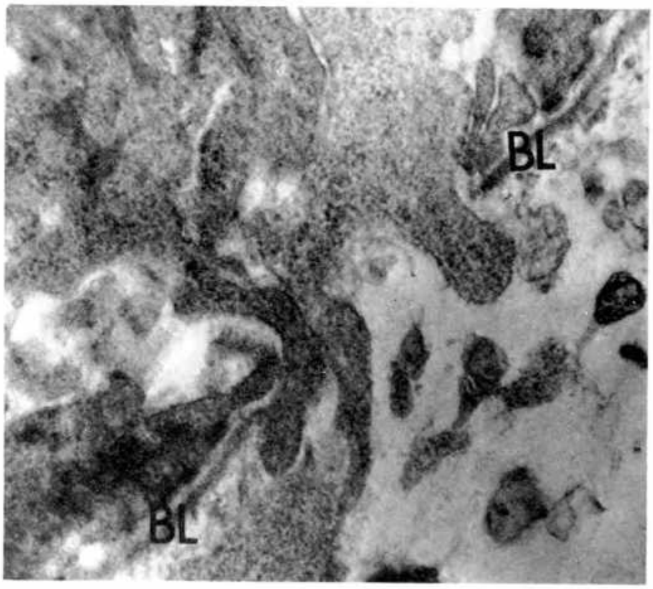

FIGURE 8. Enlargement from Fig. 7 showing hole in basal lamina (BL) (× 35,500).

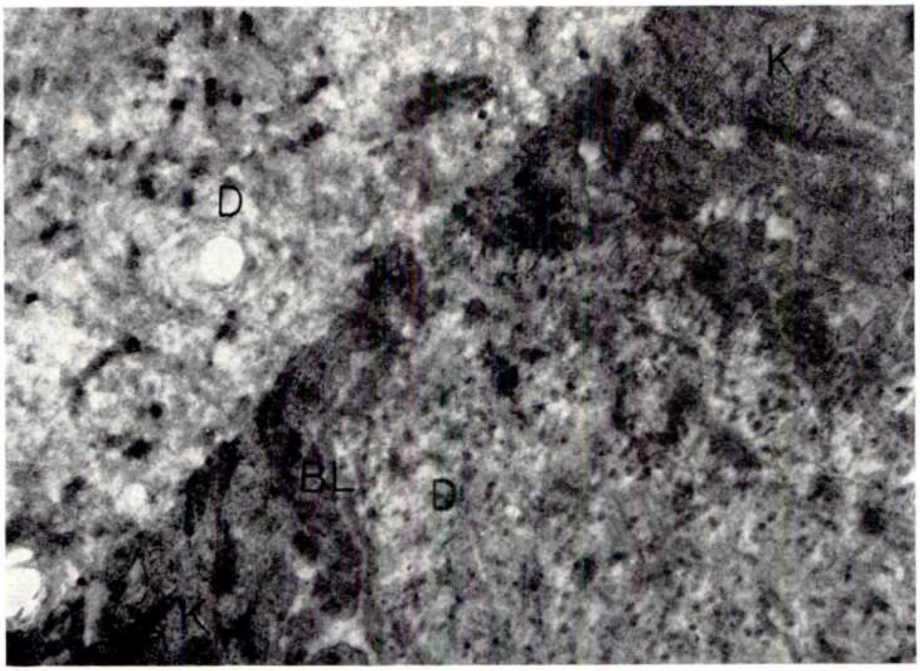

FIGURE 9. Disrupted basal lamina (BL) with degenerate dermal material (D) on either side. Degenerate keratinocytes (K) and hemidesmosomes (H) (× 18,000).

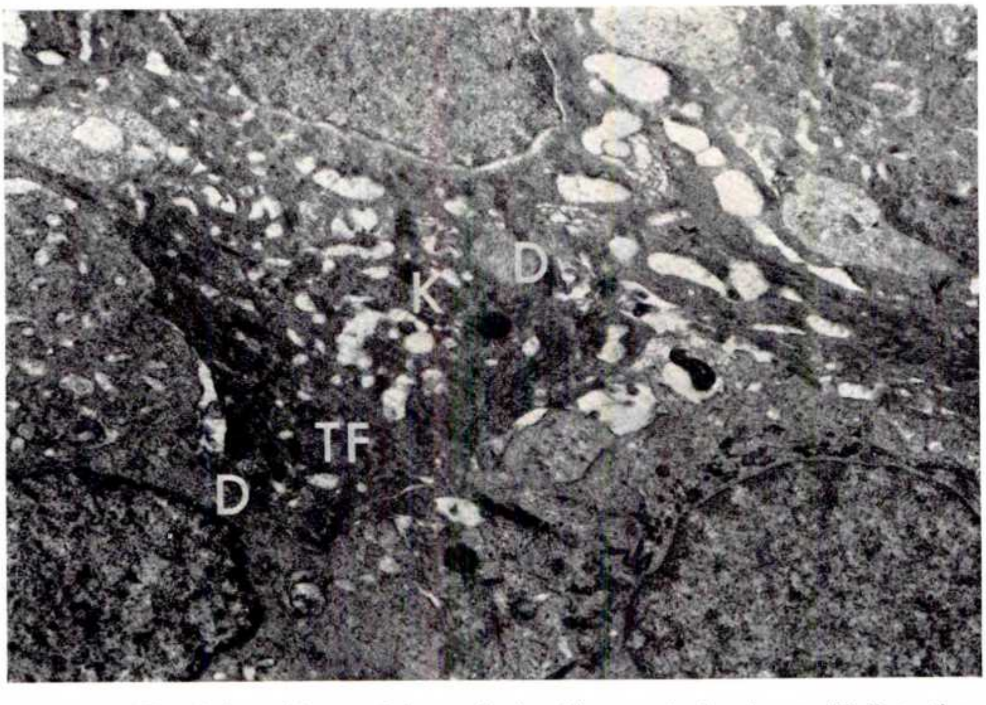

FIGURE 10. Vacuolation and increased electron density of degenerating keratinocyte (K). Retraction of tonofilaments (TF) from desmosomes (D) (× 5000).

basal lamina (Fig. 6). Normal hemidesmosomes were present adjacent to the thickened lamina, but not where the lamina was missing.

Some holes in the basal lamina had cells passing through them, partly in the epidermis and partly in the dermis. These were Langerhans' cells (Fig. 7), or unidentified 'clear cells' (i.e. without Langerhans' granules or melanosomes). Degenerate dermal material containing clumps of collagen and elastin was also seen apparently passing through some of these holes in the basal lamina into the epidermis, where it was sometimes present in large amounts, but less dense than in the dermis (Fig. 9). It had sometimes sheared through basal and suprabasal keratinocytes, leaving a part of these cells above and a part below the material. Where the epidermis was intact the most pronounced changes in keratinocytes were in the basal and suprabasal layers. These keratinocytes showed varying degrees of degenerative change, with shrinkage, vacuolation, and increased electron density (Fig. 10). Keratinocytes in the Malpighian and granular layers appeared normal. Occasional sections showed intraepidermal macrophages, and other cells which could not be positively identified and had presumably infiltrated the epidermis.

In sections from active lesions, melanocytes could not be seen, but Langerhans' cells of normal morphology were present in apparently increased numbers from the basal zone upwards. In sections from resolving lesions there were fewer Langerhans' cells; melanocytes were present in small numbers and they usually showed degenerative changes. The more active the lesion clinically, the fewer melanocytes and the more Langerhans' cells were present, the latter seeming to replace the former in the stratum basale.

DISCUSSION

The most striking clinical and light microscopic changes in LSA are dermal, and these correlate well with the EM changes seen in the present study, and with findings by other authors (Forssmann *et al.*, 1964). Epidermal changes are also present.

Our study suggests that the major abnormality is in the degeneration and reformation of collagen. Active lesions, as defined, show considerable degeneration of collagen fibres, with marked variability of fibre diameter, and apparently empty collagen tubes. Resolving lesions show much new collagen fibril formation, which is laid down irregularly. Forssmann *et al.* (1964) noted the variability in fibre diameter and 'neofibrillogenesis', and considered LSA to be primarily a disease of new collagen formation, where compaction of new collagen strangulates other dermal structures, leading to a paucity of blood vessels and fibroblasts. The descriptions would suggest that they studied resolving lesions in their two cases. Light microscopic studies also support the occurrence of new collagen formation (Steigleder & Raab, 1961).

The large clumps of elastin present throughout the diseased dermis in our cases were not noted by Forssmann *et al.* (1964) in their EM study. Most descriptions of the light microscopic features of LSA state that elastic tissue is usually absent, or present in only small amounts (Steigleder & Raab, 1961; Montgomery, 1967; Milne, 1972), and it seems possible that the disease process may alter the staining properties of elastin.

Forssmann *et al.* (1964) mentioned thickening of the basal lamina; this finding was consistently present in all our specimens. But they did not report holes in the basal lamina, its absence in places, and its replication in other areas. Basal lamina replication has been reported in various skin conditions, such as psoriasis (Cox, 1969), pityriasis rubra pilaris (Cruickshank & Mann, 1971), and porokeratosis of Mibelli (Abdel-Aziz *et al.*, personal communication, 1972) and when hyperplasia of the epidermis is produced by hexadecane (Cowan & Mann, 1971). This is probably non-specific.

Melanocytes were not seen in active lesions, but were present in small numbers, and showed degenerative changes, in resolving lesions. This would account for the pearly white appearance of active lesions and the darker colour of resolving ones. These cells seemed to be replaced by Langerhans' cells in the active lesions, and one such cell was seen part in the epidermis and part in the dermis. It has been suggested that there is a reciprocal relationship between the numbers of melanocytes and basal Langerhans' cells in the epidermis. This has been discussed in relation to vitiligo (Wolff, 1972), and the hypopigmented lesions of leprosy (Job, Nayar & Narayanan, 1972). This view is supported by our findings in LSA, but its significance is unknown.

In this study degenerate collagen appeared to be phagocytosed by dermal fibroblasts and pass together with other dermal debris, including elastin, into the body of the epidermis. Thus the skin appeared to eliminate a diseased component. The existence of transepidermal elimination of dermal material is well described, the following constituents being subjected to this process on occasions—elastic fibres (both intact and degenerate), collagen, calcium, bone, amyloid, lepra bacilli, lymphoma cells, and erythrocytes (Mehregan, 1968). In our own cases, the dermal debris was seen only within the epidermis, but this does not exclude the possibility that it might be eliminated through the skin surface. This could account in part for the small punctate lesions which clinically have been called follicular hyperkeratosis, although it is known that histologically true follicular hyperkeratosis may occur.

In contrast to Forssmann's observations, collagen degeneration appeared as prominent as 'neofibrillogenesis', casting doubt on the primary role of the latter. The reduction in the number of capillaries is in agreement with Forssmann's findings, but we would hesitate to attribute this to either collagen degeneration or 'neofibrillogenesis'.

All the evidence suggests that the epidermal changes are secondary to those in the dermis.

ACKNOWLEDGMENTS

We thank Dr H.J.Wallace for permission to study Cases 3 and 4, and Dr C.N.D.Cruickshank for his help with preparation of the paper. Fig. 2 is reproduced by courtesy of the Editor of *Transactions of the St John's Hospital Dermatological Society*.

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Protein-losing enteropathy in dermatitis herpetiformis

ANTHONY D.TOFT, ROBERT C.HEADING AND JOHN SIMPSON

Departments of Therapeutics and Medical Physics,
The Royal Infirmary, Edinburgh EH3 9YW

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SUMMARY

A patient is described with gross intestinal protein loss occurring in association with a typical dermatitis herpetiformis enteropathy, in whom no other gastrointestinal abnormality was identified.

The jejunal mucosa is abnormal in 95% of patients with dermatitis herpetiformis (Brow *et al.*, 1971). Although the intestinal lesion is similar to that found in coeliac disease, both in histological appearance (Marks, Shuster & Watson, 1966) and in its response to a gluten-free diet (Shuster, Watson & Marks, 1968; Fry *et al.*, 1968), laboratory evidence of malabsorption is remarkably infrequent (Fry *et al.*, 1967) and symptoms of intestinal dysfunction are rare. In this communication we describe a patient with dermatitis herpetiformis enteropathy who developed hypoproteinaemic oedema due to intestinal loss of plasma protein. It appears that only two similar cases have been previously reported (Gjone & Oyri, 1970).

CASE REPORT

A coal miner aged 57, who had had dermatitis herpetiformis for 15 years, presented in August 1970 with generalized oedema. Although his serum albumin was 2·3 g/100 ml, the urinary excretion of protein was consistently less than 2·0 g/day. Microscopy of the spun deposit of urine showed increased numbers of red blood cells. He was normotensive. The antistreptolysin O titre was normal, but the serum cholesterol was 428 mg/100 ml, and the erythrocyte sedimentation rate (ESR) was 107 mm in the first hour (Westergren). Light and electron microscopy of a renal biopsy specimen demonstrated a resolving proliferative glomerulonephritis. The patient recovered spontaneously and on discharge the serum albumin had risen to 3·8 g/100 ml, and there was neither proteinuria nor haematuria.

In February 1971 he developed a recurrence of generalized oedema whilst his urine remained protein-free. On re-investigation the following results were obtained: serum albumin 3·1 g/100 ml; serum cholesterol 498 mg/100 ml; Hb 13·3 g/100 ml, and ESR 110 mm in the first hour (Westergren); serum folate 3·1 ng/ml (normal 2·5–18 ng/ml); red cell folate 200 ng/ml of packed cells (normal 79–374 ng/ml of packed cells); and serum vitamin B_{12} (Euglena) 180 pg/ml (normal 150–1000 pg/ml). A folate absorption test was normal, 2·82 mg of folate appearing in the urine in the 24 h after administrating 5 mg folic acid subcutaneously, and 2·87 mg appearing in the urine in the 24 h after the same oral dose. Serum iron was 67 μg/100 ml, and total iron binding capacity 246 μg/100 ml. Bone marrow examination showed normoblastic erythropoiesis with no stainable iron. Serum immunoglobulins were normal. Liver function tests, including bromsulphthalein excretion test gave normal results. Faecal fat excretion was 4·5 g/day (mean of 16 days), and 5·9 g D-xylose appeared in the urine in the 5 h after 25 g had been given orally. Repeated tests for occult blood in the faeces were negative. Radiological studies included a barium meal and small bowel follow-through examination, barium enema and abdominal lymphangiography; all of which gave negative results. The findings on sigmoidoscopy were normal. A jejunal biopsy taken with a Crosby capsule approximately 10 cm distal to the duodeno-jejunal junction showed subtotal villous atrophy. Gastrointestinal protein

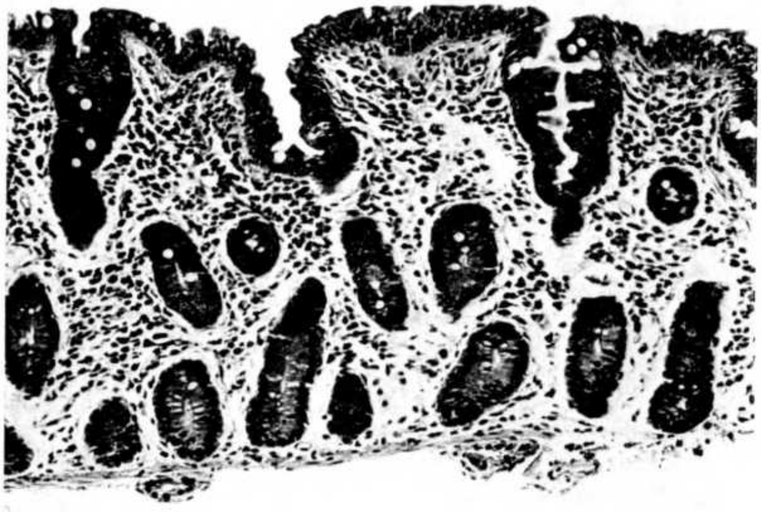

FIGURE 1. Partial villous atrophy in jejunal biopsy taken 10 cm distal to the duodeno-jejunal flexure after 5 months of gluten-free diet (H & E, × 140).

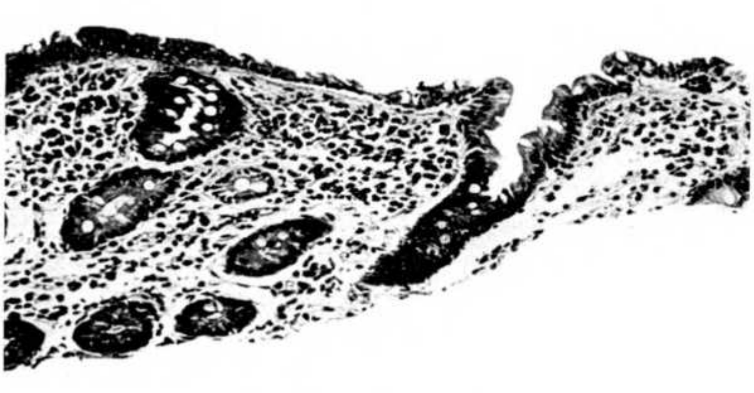

FIGURE 2. Subtotal and partial villous atrophy in another field of same jejunal biopsy specimen (H & E, × 140).

loss was assessed using ^{51}Cr-chloride (Rubini & Sheehy, 1961), and 5·7% of the intravenously administered radioactivity appeared in the faeces over 5 days (normal value <1%), representing a daily faecal loss of 8 g plasma albumin.

The patient was treated with a gluten-free diet in an attempt to reverse the protein loss. After 5 months on this diet he still had periorbital oedema, his serum albumin was 2·1 g/100 ml, and there was no recurrence of proteinuria. Two further jejunal biopsies, taken at approximately 5 and 10 cm distal to the duodeno-jejunal flexure showed partial villous atrophy, and a combination of subtotal and partial villous atrophy respectively, (Figs. 1 and 2). On this occasion 4·8% of the intravenously administered chromium isotope appeared in the faeces over 5 days, corresponding to a daily faecal loss of 4 g of plasma albumin.

DISCUSSION

Loss of plasma protein into the gastrointestinal lumen has been observed with inflammatory conditions of the gastrointestinal mucosa, in disorders of the alimentary lymphatics, and with several gastrointestinal tumours (Waldmann, 1966). In this patient, gross intestinal protein loss has occurred in association with an otherwise typical dermatitis herpetiformis enteropathy, and no other gastrointestinal abnormality has been identified. The episode of glomerulonephritis possibly contributed to the patient's initial development of oedema, but clearly cannot account for the persistent hypoproteinaemia.

Although intestinal villous atrophy is almost invariably present in dermatitis herpetiformis, clinically significant loss of plasma protein from the lesion is exceptionally rare. The intestinal abnormality is seldom extensive, and the absence of biochemical evidence of malabsorption indicates that much of the small bowel is unaffected (Brow *et al.*, 1971). It is, therefore, remarkable that in this patient, and in the two similar cases previously reported (Gjone & Oyri, 1970), the clinical and laboratory findings suggest that this type of restricted lesion has been responsible for the loss of plasma protein. This contrasts with comparable protein loss due to adult coeliac disease, where evidence of malabsorption is usually obtained. It has been suggested that plasma protein loss in coeliac disease results from exudation due to the intestinal lymphatic dysfunction caused by villous atrophy (Kalima, 1971), and it has been shown that major protein loss may be diminished by a gluten-free diet (Rotem & Czerniak, 1964). In our case of dermatitis herpetiformis enteropathy, this form of dietary treatment produced some reduction in protein loss, but the patchy nature of the lesion precluded a valid biopsy assessment of response to treatment. In one of the patients reported by Gjone & Oyri (1970), treatment with a gluten-free diet had no apparent effect on intestinal protein loss, although it corrected co-existing steatorrhoea. It is of interest that in two of the three cases of protein-losing enteropathy in dermatitis herpetiformis now documented, the serum cholesterol has been markedly elevated, in contrast to the subnormal levels usually found in coeliac disease.

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Indirect immunofluorescence testing in bullous diseases

M.D.TROTTER

MRC Unit for Research on the Experimental Pathology of the Skin,
The Medical School, The University, Birmingham 15

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SUMMARY

Sera from patients with bullous pemphigoid, pemphigus vulgaris and other bullous conditions have been tested for auto-antibodies by the indirect immunofluorescence (IIF) method.

Positive confirmation of the initial diagnosis was obtained in approximately 75% of both pemphigus and pemphigoid cases.

It is suggested that the major source of error in the IIF test lies in the existence of 'pemphigus-like antibodies'; and clinically, in diagnosing some cases of dermatitis herpetiformis as pemphigoid.

The contributions of Beutner and his associates to our knowledge of the aetiology of pemphigus vulgaris and bullous pemphigoid as 'auto-immune' skin diseases are so extensive as to leave few questions remaining in terms of technique, and have gone far towards rationalizing their findings (Beutner, Jordan & Chorzelski, 1968; Beutner, Chorzelski & Jordan, 1970).

We have been concerned, therefore, to try to assess the value of these techniques in terms of everyday clinical usage and to discover where errors, diagnostic on the part of the clinician, and investigative on the part of the laboratory, might be found, and consequently, where the one might act as a corrective of the other.

THE PATIENTS

Twenty-one different clinicians have sent 187 samples of sera (including replicates) for indirect immunofluorescence testing (IIF). Serum was taken from patients who were suffering from pathological skin conditions which were bullous or blistering in nature and which might conceivably have some features in common with pemphigus vulgaris or with bullous pemphigoid.

These patients have been divided into categories on a basis of the clinicians' diagnosis where this was clear, or into broader categories where the diagnosis was in question, or where a differential diagnosis was offered.

Fifty sera, excluding replicates, were obtained from patients with bullous pemphigoid; twenty-seven from patients with pemphigus vulgaris or foliaceous, and twenty-eight from patients with bullae that suggested one or other of these conditions, at least as a possible differential diagnosis, which might be excluded or confirmed by the immunofluorescence test.

Other conditions categorized were dermatitis herpetiformis (eleven); discoid or systemic lupus erythematosus (thirteen); epidermolysis bullosa (eleven); unspecified blisters and bullae (ten); oral blisters and ulcers (five); mucous membrane pemphigoid (four); systemic sclerosis (five); vasculitis (four); erythema multiforme (four); bullous lichen planus (two); and numerous other defined conditions in ones or twos. Generally speaking, the diagnosis in these groups was not in doubt, or at least a diagnosis of pemphigus or pemphigoid was not considered a possibility.

METHODS

Frozen sections of guinea-pig lip (7 μm thick) were cut using a Frigistor (De La Rue Frigistor Ltd) cooled microtome and stored at −20°C until used. Storage was generally limited to 1 week. Monkey oesophagus strips were immersed in OCT compound in foil cylinders and quick frozen over liquid nitrogen. The more friable oesophageal tissue necessitated the cutting of thicker sections (12–15 μm).

For staining, sections were air dried, covered with a few drops of 1/10 serum and incubated at room temperature for 30 min. After washing (10 min × 3) in 'Bacto' F.A. buffer and draining, they were incubated with a few drops of fluorescein-labelled sheep anti-human IgG (Wellcome), usually 1/10 dilution, for 30 min, washed as before, and finally mounted in 'Bacto' F.A. mounting fluid.

Chessboard titrations with 1/10–1/640 serum and 1/10–1/40 antisera were initially carried out to assess antibody titre and to find satisfactory dilutions, but subsequently, when it was apparent that a positive or negative result only was required, numbers were reduced by using a standard 1/10 antisera (antibody protein of original solution 0·39 mg/ml, F/P ratio OD 0·79 or Molar 3·6). Dilutions of sera used were reduced to 1/10 and 1/50, and ultimately to 1/10 only, when no failure of reaction due to 'pro-zone phenomenon' was observed, although that possibility cannot be excluded throughout.

Sections were examined, using dark ground illumination (UV excitation of about 365·5 nm, from a 200 W HP mercury lamp) on a Nikon fluorescence microscope, for evidence of labelled antibodies.

RESULTS

Dermatitis herpetiformis

Sera from eleven patients (four male and seven female) who were considered clinically, pharmacologically, and in general histologically to have unequivocal DH with few atypical features, were tested and found to give negative IIF tests using conjugated anti-IgG and guinea-pig lip substrate. The mean age of the group was 49 (15–86). Five other cases which were shown ultimately to be DH were similarly negative with respect to IIF tests, but as they had been submitted to tests for differential diagnosis and they may have had atypical features.

Lupus erythematosus

Sera from thirteen patients (one male and twelve female) who were regarded as unequivocal cases of LE or SLE, were tested and found to be negative with respect to basement membrane and intercellular fluorescence. The mean age of the group was 53 (26–69).

Three showed strong anti-nuclear fluorescence and a further three some lesser degree of ANF.

Three further cases, where LE was a possible differential diagnosis, were negative in all respects; another case, diagnosed as pemphigoid, showed strong ANF, but remained categorized clinically as pemphigoid.

Epidermolysis bullosa

Sera from ten cases of various types were examined and found negative for basement membrane and

TABLE 1. 'Pemphigus' group

Diagnostic group	No. of patients	Intercellular fluorescence
Unequivocal pemphigus vulgaris	14	12
Pemphigus foliaceous	5	2
Pemphigus erythematosus	1	1
Oral lesions, pemphigus ?	2	1
Pemphigus/pemphigoid	3	3
Initial diagnosis pemphigoid	2	2
Total	27	21

intercellular fluorescence. One showed anti-nuclear fluorescence, but this could not be found in other members of the same family, or indeed in any other case, and must be regarded as coincidental.

Several cases of erythema multiforme, mucous membrane pemphigoid, bullous lichen planus, vasculitis and other forms of bullae have been examined with negative results.

Pemphigus vulgaris or foliaceous

A total of twenty-seven patients (eleven male, sixteen female; mean age 59, range 34–86) were placed in the pemphigus category on a basis of clinical diagnosis and/or immunofluorescence findings.

This group has been subdivided in Table 1, and the corresponding number of positive intercellular (IC) IIF tests shown. Three of these were referred as either pemphigus or pemphigoid, and showed positive IC fluorescence, so that the former seems more probable. Two cases, with a preliminary diagnosis of bullous pemphigoid, showed intercellular fluorescence and subsequently were shown to be pemphigus vulgaris on a clinical and histological basis also. Two cases had oral lesions only: (a) male, aged 68—pemphigus of mouth. Negative fluorescence. (b) female, aged 55—oral lesions: positive, though weak, intercellular fluorescence on all occasions tested. The diagnosis was not agreed between clinicians.

Of the whole group, therefore, twenty-one out of twenty-seven showed some degree of intercellular fluorescence (77%).

Bullous pemphigoid

A total of fifty patients (twenty-three male, twenty-seven female; mean age 72, range 55–90) were placed in the 'pemphigoid' category on a basis of clinical diagnosis. Positive fluorescence of the basement membrane was found in this series only where there was a diagnosis of pemphigoid. Of these, forty-two (twenty male, twenty-two female; mean age 72) were regarded as unequivocal pemphigoid, i.e. the clinician was in no doubt and there were no contradictory features other than negative basement membrane fluorescence in some cases.

All 'pemphigoid' sera were tested with both guinea-pig lip and monkey oesophagus substrates,

TABLE 2. Incidence of basement membrane fluorescence in forty-two undisputed cases of pemphigoid

Guinea-pig lip			Monkey oesophagus		
+	Weak +	–	+	Weak +	–
24	8	10	29	6	7

the latter giving a slightly higher percentage of positive results than guinea-pig (76% and 83% respectively), as shown in Table 2. Three sera which were negative when using guinea-pig lip were clearly positive on monkey tissue, and two sera weak or doubtful on guinea-pig lips also became positive on monkey tissue. There was no detectable reason for this change.

The six out of forty-two cases which were described by the clinician as undoubtedly pemphigoid, but where basement membrane fluorescence could not be detected on either guinea-pig or monkey tissue substrates, are described in more detail: Biopsy +. signifies histological compatibility with a diagnosis of pemphigoid. The duration of the disease is given when known.

1. *Female 79*. Biopsy +. 6 years. Controlled, prednisone 5 mg: also present were intertrigo, oral blisters, keratosis of fingers and leiomyoma.

2. *Male 78*. Biopsy +. 2 months. Controlled, betnovate and synthetic depot ACTH. Oral and local lesions, thyroid tumour.

3. *Female 65*. Biopsy +. Controlled, 5–10 mg prednisone. Developed sarcoidosis after 1 year.

4. *Female 68*. Biopsy +. 2 years. Controlled 5 mg prednisone.

5. *Female 78*. Biopsy +. 3 years. Off steroids 8 months—recurrence of blistering, 5–10 mg prednisone.

6. *Female 70*. Biopsy +. Controlled ACTH.

It may be noted that two of these were controlled by relatively modest steroid therapy, and that three whose duration of disease was known were 'old'. cases. Two had some form of tumour, and another developed sarcoidosis, which might imply a degree of immunological atypicality.

The remaining eight out of fifty, where the diagnosis was originally pemphigoid, but where subsequent events gave rise to doubt, are also described in some detail. The reassessment is the clinical view.

1. *Male 70*. Biopsy +. 2 years. Considered typical pemphigoid. Negative BM fluorescence but strong fluorescence of dermal collagen fibres. Re-assessment: pemphigoid.

2. *Female 65*. Biopsy +. 4 weeks. Annular lesion with blisters at edge of abdomen. Negative BM fluorescence. Now responding to dapsone. Re-assessment: dermatitis herpetiformis.

3. *Male 70*. Died. Limited data available. Negative BM; positive intercellular fluorescence. Re-assessment: probably pemphigus vulgaris.

4. *Female 66*. Biopsy +. 3 years. Free of blisters 2 years and off steroids, flare up, treated prednisone. Negative BM fluorescence, positive ANF which disappeared after 3 months. LE suggested. Re-assessment: pemphigoid.

5. *Female 62*. Biopsy +. 1 year. Negative BM fluorescence. Subsequent response to dapsone. Re-assessment: dermatitis herpetiformis.

6. *Male 22*. Biopsy inconclusive. 2 years. Initially atypical pemphigoid or DH. IIF negative. Unsatisfactory response to prednisone and dapsone/sulphapyridine. Re-assessment: possible DH, 'allergy ?', inconclusive.

7. *Female 69*. Biopsy unsatisfactory. New. Initial diagnosis pemphigoid, BM fluorescence negative, but IC fluorescence present. Later biopsy suggested pemphigus. Re-assessment: pemphigus vulgaris.

8. *Female 49*. Biopsy +. New. Negative BM fluorescence, treated with prednisone, subsequently showed response to dapsone. Re-assessment: dermatitis herpetiformis.

In the eight patients whose diagnosis of pemphigoid was, in the light of immunofluorescence and subsequently other tests, clearly controversial, one (No. 6) was always, and remains, controversial. Three were later shown to be responsive to dapsone, and may thus be labelled dermatitis herpetiformis (Nos. 2, 5 and 8). Clinical re-assessment was undoubtedly precipitated by IIF findings, but the final diagnosis of DH was eventually confirmed by a therapeutic test and perhaps some degree of clinical 'atypicality'.

Two further cases were re-diagnosed as pemphigus vulgaris and subsequently agreed clinically, following the finding of IC rather than BM fluorescence, which would have been expected in pemphigoid (Nos. 3 and 7).

The two remaining cases (Nos. 1 and 4) both showed abnormal features of immunofluorescence—fluorescent collagen fibres in one (No. 1) and fluorescent nuclei in the other (No. 4). In both, the original diagnosis of pemphigoid is maintained by the clinician, but the incongruous IIF results must leave an element of doubt.

There was thus a proven error of diagnosis in five out of fifty (10%) of clinically diagnosed bullous pemphigoid, rising to a possible 15% approximately, if more dubious, but not disproven, diagnoses are included. Alternatively, the clinician was 90% correct!

IIF in differential diagnosis

Excluding firm diagnoses of pemphigus, pemphigoid, LE, DH, etc., from the total number of sera tested, there remained twenty-seven sera where the diagnosis was in doubt, and where clarification might be obtained through IIF testing.

One of this group showed positive IC fluorescence at a titre of 1/320, indicating a diagnosis of pemphigus, and another three showed doubtful, low titre IC fluorescence or 'pemphigus-like' antibodies, which was taken as a suggestion to the clinician that pemphigus should not be excluded without further evidence. No sera gave a positive reaction for basement membrane antibodies, and thus no unsuspected cases of bullous pemphigoid were found.

The function of the test in these cases was therefore primarily exclusive, rather than positively diagnostic, and also served to differentiate bullous lichen planus from the association of pemphigoid and lichen planus, or to differentiate erythema multiforme from bullous pemphigoid.

DISCUSSION

In evaluating the IIF test as an aid to the clinician, it is necessary to examine the premises on which this is based.

Clinical diagnosis has been used here in order to categorize individual patients into groups, so that a degree of subjective impression is inevitable, but in general the diagnosis was categorized within its specific disease group only where there was a high degree of conviction on the part of the clinician and usually with some supporting evidence, such as compatible histology, repeated therapeutic response or consistent immunofluorescence.

For geographical reasons, and in order to minimize the time and trouble expended by the clinician in taking, preserving and sending pathological specimens to the servicing laboratory, direct immunofluorescence tests were not carried out. In consequence, the criterion of a positive direct test as a decisive feature of diagnosis in the case of pemphigus and pemphigoid could not be used, and categorization had to be carried out on a purely diagnostic basis (Chorzelski & Beutner, 1972).

Guinea-pig lip was the substrate of choice on grounds of availability and clarity of histology, but replicate testing on pemphigoid sera and using monkey oesophagus sections showed a slightly increased incidence of positive IIF reactions. This advantage was outweighed by the clearer histology of guinea-pig lip.

It has been shown (Chorzelski & Beutner, 1969, 1972; Thivolet & Bayvin, 1972) on several occasions that the IIF test can yield negative results even when the diagnosis is not in doubt, i.e. when the direct test is positive. The rate of failure is usually about 25%, less if primate tissue is used, and according to Thivolet & Bayvin may be as low as 8% if a diagnostic correction is made on a basis of the sulphone therapeutic test. The present results show that an appreciable number of cases diagnosed pemphigoid are in fact dermatitis herpetiformis, and a failure to detect antibodies should be regarded as grounds for reconsideration, of a diagnosis of pemphigoised, with perhaps a therapeutic test, particularly if the clinical picture is not entirely clear.

If, however, clinical and laboratory diagnoses agree and are positive, there can be little doubt that the diagnosis was initially correct.

In the case where IIF tests are positive but not in agreement with the clinical diagnosis, one or the other must be wrong. If the basement membrane or the intercellular fluorescence is strongly positive, it is unmistakeable; the clinician must reconsider his diagnosis, then preferably repeating the IIF test with a fresh sample of serum.

Equally, the absolute negative is not difficult to interpret, but as has been shown, this is a result, which offers neither confirmation nor contradiction of diagnosis with certainty.

There does exist, however, a degree of fluorescence, usually of the intercellular type and of low titre, which is difficult to interpret, and where errors of interpretation may occur (Anderson *et al.*, 1970; Burnham & Fine, 1972; Beutner, 1971; Newcomer, 1970). Foreknowledge of these 'pemphigus-like antibodies' and the application, where practicable, of Beutner's 'house rules' may minimize, but are unlikely to eliminate, all errors.

The question remaining is whether IIF testing is of real value to the clinician. The answer must be that in some cases it is, both in terms of confirming a diagnosis and in sometimes questioning a diagnosis made clinically. It is apparent that some reservations must attach to the firm diagnosis of bullous pemphigoid until further confirmation is made by IIF testing, or by the elimination of the possibility of DH through therapeutic testing. Honeyman *et al.* (1972) have commented similarly on this occasional confusion between DH and pemphigoid, and they suggest that there is an overlap between these conditions.

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The action spectrum in polymorphic light eruption

W. FRAIN-BELL, A. DICKSON, JOYCE HERD AND I. STURROCK

Department of Dermatology, University of Dundee

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SUMMARY

A study of fifty-six cases of polymorphic light eruption has shown that the action spectrum frequently lies in both the long ultraviolet wavelengths (320–400 nm) and the short ultraviolet wavelengths below 320 nm.

Despite an increasing interest in the photobiological aspects of skin reactions, little is known of the factors responsible for polymorphic light eruption (PLE). It is important to define as clearly as possible the clinical characteristics associated with the action spectrum of this relatively commonly diagnosed condition. The meaningfulness of action spectrum studies is dependent on the prior recognition of a defined clinical entity.

CLINICAL FEATURES

Although this report is concerned with the action spectrum, it is necessary for comparison with the results obtained by other investigators to define the clinical presentation of the subjects studied. We have excluded all cases of children with vesicular/pruriginous lesions followed by pitted scarring, i.e. 'summer prurigo/prurigo aestivale'. Reference to Table 1 shows that of the fifty-six patients the majority were females (91%), the abnormal response to light having appeared for the first time in childhood (50%) or, at least in the majority of cases, before the age of 20 years (73%). The changes in the skin following exposure to light consisted of a variable admixture of erythema, oedema, with papules, weals, and later pruriginous lesions and even lichenification. Although in the majority of cases of PLE these changes were confined to exposed skin sites, this was not always so, covered areas

TABLE 1. Details of patients

No. of cases	Female	Male
56	51	5

Age of onset (years)	0–10	11–20	>20
No. of patients	28	13	15

being occasionally involved, although in some instances this could have been explained by irradiation through thin clothing. The incidence of affection of exposed sites may be variable and the reason for this is not always clear; for instance there may be in one case maximum affection of all exposed skin, in another case relative sparing of the face. In this group of fifty-six cases of PLE, seventeen (30%) improved to a certain extent with the advancement of the summer. The majority start in the spring, experiencing active skin responses right through until the autumn, apparently related in the main to the degree of sunlight exposure. Sixteen of the patients also had some affection of exposed skin sites during the winter months. In three patients this winter affection could have been explained on the basis of an atopic prurigo or eczematous reaction, and in one case as a manifestation of rosacea. In the remaining twelve, however, there did not appear to be any satisfactory explanation other than exposure to light. Particular reference was made by some of these twelve patients to reactions of their skin following exposure to winter sunshine. Although there is no doubt the condition can improve in later adult life, this improvement with age being noted in ten of the fifty-six cases, it frequently remains active and troublesome well into middle age. None of these patients appeared to have been exposed to photoactive substances arising either from systemic disease or its treatment, or from environmental contact.

MATERIALS AND METHOD

Action spectrum studies were carried out in the main on consecutive patients, seven being excluded on account of incomplete results. The skin (size of irradiated area = 8 mm diameter) of the back was

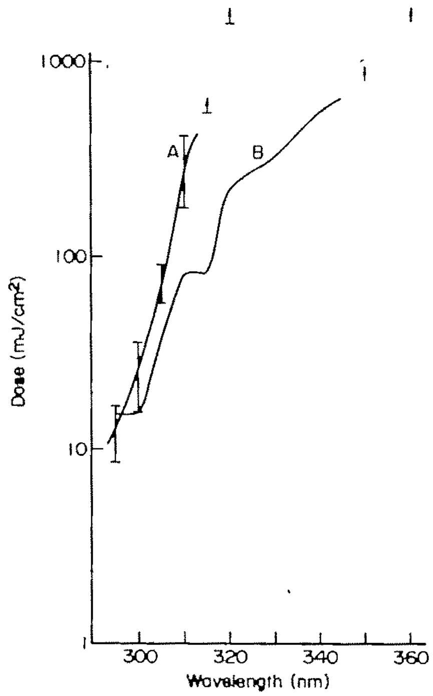

FIGURE 1. Curve A is that for the mean MED in seventeen normal subjects with the vertical bars showing one standard deviation about the mean. Curve B is the line drawn through the minimum papular response dose values in a case of polymorphic light eruption. The arrows indicate that the MED was not reached at these wavelength peaks.

irradiated using a 1600 W xenon arc light source. This was passed through a grating monochromator (Bausch and Lomb 33-86-40-45, 600 lines/mm, reciprocal dispersion 3·3 nm/nm slit width). Schott filters (2·5 mm thick), such as WG305 at 365 nm, WG345 at 400 nm, were used to minimize scatter. The energy output was measured prior to each exposure by means of a thermopile (Hilger Schwartz FT4), coupled to a Solartron digital voltmeter (LM14202), and expressed as milliwatts per centimeter squared (mW/cm^2) (Mackenzie & Frain-Bell, 1973). The response of the skin was graded as follows: 0, no response; $\frac{1}{2}$, doubtful erythema; 1, minimal but definite erythema; 2, marked erythema; 3, erythema plus oedema. The morphology was recorded as that of either uniform erythema or erythema with a papular reaction. Readings were carried out at 7, 24, and if necessary 48 h after irradiation. Where necessary, bigger doses (i.e. multiples of a previously defined minimal erythema dose) were given so as to demonstrate possible abnormal reactions. Initial screening was carried out using the following broad wavebands: 290–320 nm; 305–365 nm; 335–400 nm; 370–430 nm; 430–490 nm; 470–530 nm. This was followed by 10 nm wavelengths bandwidths from 290–360 nm, i.e. 290–300; 295–305; 300–310; 305–315; 310–320; 315–325; 320–330; 325–335; 335–345; 340–350; 345–355; 350–360 nm.

RESULTS

In the fifty-six cases studied, the results were based on full initial broad band and subsequent narrow band pass irradiation programme in forty-eight (Figs. 2 and 3), and to broad band pass irradiation in only eight.

Fig. 1 is an example of the type of action spectrum for abnormal papular responses which was obtained. It can be seen that it stretches from 290 nm to include wavelengths within the

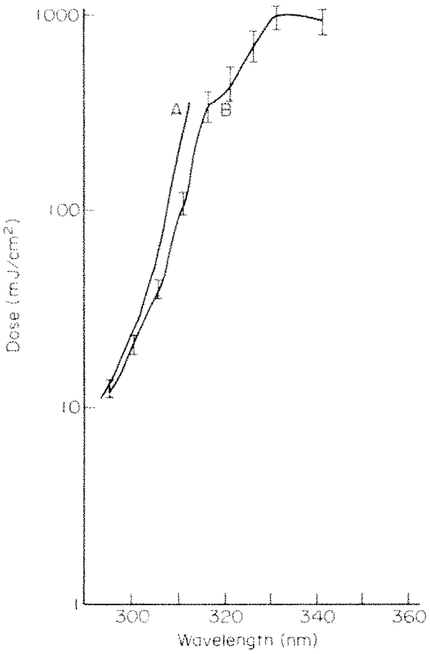

FIGURE 2. Curve A is the same as that shown in Fig. 1 with the standard deviation removed for clarity. Curve B is the curve for the mean minimal response dose values found in forty-eight cases of polymorphic light eruption, the bars being one standard error about the mean.

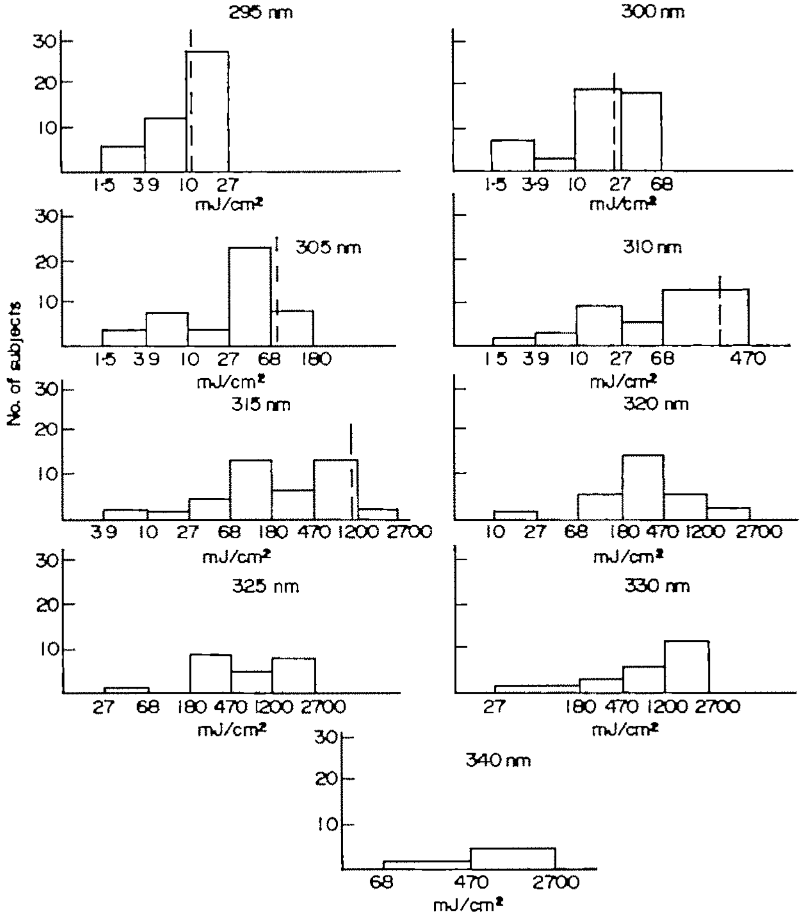

FIGURE 3. The histograms show the relationship between the range of the minimal dose for an abnormal response and the number of patients affected at given wavelengths between 295 and 340 nm. The mean MED in normal subjects is shown by the broken vertical bar.

TABLE 2. Relationship of abnormal papular responses in PLE patients to minimal erythema dose (MED) in seventeen normal subjects at 290–320 nm

No. of cases	< MED	> MED	> or < MED	Same as MED	Doubtful result†
56	19	16	8*	5	8

* In the eight patients referred to in this column the papular response occurred with dose levels less than that required to produce minimal erythema in normal subjects only at some of the wavebands.

† The 'doubtful result' column refers to those patients in whom there was doubt as to the accuracy of a papular response recording.

TABLE 3. No. of cases responding for each wavelength peak and the minimal dose required to produce that response

	Wavelength (nm)								
Radiation dose (mJ/cm²)	295	300	305	310	315	320	325	330	340
1·5–3·3	6	7	3	1	—	—	—	—	—
3·9–8·2	12	3	7	3	2	—	—	—	—
10 –22	27	20	4	9	1	2	—	—	—
27 –56	—	18	23	5	4	—	1	1	—
68 –150	—	—	8	13	13	5	—	1	1
180 –390	—	—	—	14	7	14	8	3	1
470 –1000	—	—	—	—	13	6	5	6	4
1200 –2700	—	—	—	—	2	5	8	11	4
Total no. of cases reacting at waveband	47	48	45	45	42	32	22	22	10

short UV (i.e. <320 nm) and in the long UV spectrum, as far as 340 nm. Of the fifty-six cases studied, only fifteen (27%) had an action spectrum which appeared to be confined to wavelengths below 320 nm. In Figs. 2 and 3, a composite of the data from forty-eight of the patients studied shows the broad spread of the values of this spectrum. These values refer to minimal dose for abnormal papular responses and/or erythema. The number of patients reacting with abnormal papular responses at each wavelength peak is shown in Table 3. It was found that, contrary to previously held views, in many cases (53%) these abnormal skin responses could be produced with dosage levels less than that required to produce minimal erythema in all normal subjects tested (Table 2). In nineteen out of the fifty-six subjects, i.e. 34%, this abnormal papular response occurred at all wavelengths throughout the action spectrum. In eight, i.e. a further 14%, giving a total of 48%, this abnormal papular response

TABLE 4. Minimal dose (mJ/cm²) in fourteen patients for abnormal response at 365–500 nm, except where shown by symbol <, where threshold was not ascertained

	Peak wavelength (nm)				
Patients' initials	365	400	430	460	500
J.B.	5,600	47,000	—	—	—
M.A.	< 10,000	47,000	—	—	—
D.L.	—	47,000	—	—	—
J.McC.	4,700	< 15,000	—	—	—
G.M.	< 10,000	< 82,000	—	—	—
S.B.	4,700	< 47,000	—	—	—
L.S.	5,600	47,000	—	—	—
A.M.M.	< 5,600	47,000	—	—	—
G.T.	—	< 22,000	—	—	39,000
V.T.	< 10,000	< 22,000	—	—	—
A.D.	—	47,000	—	—	—
J.McG.	—	—	82,000	—	75,000
F.B.	—	90,000	< 100,000	< 130,000	—
M.Q.	—	—	< 96,000	—	—

was produced with dose levels less than that required to produce minimal erythema with some, but not all, of these wavelengths. In fourteen patients (25%) abnormal responses occurred with irradiation from within the long UV and/or visible wavebands. In ten of these the response was that of erythema, in one case a questionable reaction which was probably urticarial (G.T.), and in three a papular reaction (A.M.M., S.B. and J.McG.). In eight of these cases these abnormal responses occurred at a wavelength peak of 365 nm (335–400 nm) as well as at 400 nm (370–430 nm), and this could presumably have been due to the long UV component in each waveband. However, in the remaining five cases, two (D.L. and A.D.) gave abnormal responses at the 400 nm peak (370–430 nm) only; one case (J.McG.) at 430 nm (400–460 nm) and 500 nm (470–530 nm); one case (F.B.) at 400, 430 and 460 nm (430–490 nm), and one case (M.Q.) at 430 nm only.

DISCUSSION

The result of the investigation of some of these patients was reported some years ago (Frain-Bell, MacKenzie & Witham, 1969) at which time irradiation was carried out using a 6 kW water-cooled high output xenon light source with appropriate cut-off filters (Turnbull, Frain-Bell & MacKenzie, 1967). The skin was exposed to broad wavebands, either in the short UV range below 320 nm or, by means of a Schott WG4 filter, from beyond this in the long UV (>320 nm). It is interesting that even with this less sophisticated technique there was a definite indication of a spread of the action spectrum into the long UV waveband, particularly in the region around 320 nm, i.e. the 'border' zone between short and long UVR. By means of the greater definition obtained by the use of a grating monochromator, it has now been possible to confirm our earlier findings and to arrive at a more accurate definition of this action spectrum (Mackenzie & Frain-Bell, 1973).

Blum (1941), in a review of the literature up to that time, stated that most of the cases of PLE responded 'abnormally to the sunburn spectrum, but in a few cases one must conclude that the sensitivity is due to other wavelengths.' Epstein (1942) and Epstein (1966) continued to support this view, the latter author finding that the action spectrum in his personal series of eighty-five patients was confined to the sunburn range (290–320 nm). Our present results support the views expressed by Wiskemann & Wulf (1959); Stevanovic (1960); Magnus (1964); and Verhagen (1966), that the action spectrum in PLE was not confined to the short UV wavelengths below 320 nm.

Studies are continuing to try to determine whether this prolongation of the action spectrum beyond 320 nm in the long UV could be explained by the presence of scattered radiation from the short UV wavelengths, but this would not pertain to the fourteen patients who reacted at 365, 400 and 500 nm. Here, appropriate Schott cut-off filters were used in conjunction with the monochromator and stray radiation from the 300 nm region was consequently insignificant.

PLE would appear, therefore, to be an idiopathic photodermatosis which affects females in the majority of instances, frequently starts in childhood and lasts for many years, and sometimes even into late adult life. The abnormal skin changes are produced by UV irradiation from wavelengths within the range from 290 to 320 nm, in many cases also by wavelengths in the long UV as far as 340 nm, and less often by visible wavelengths, such as those found between 400 and 500 nm. These abnormal skin changes provoked by wavelengths between 290 and 340 nm will frequently occur as a result of exposure to doses less than that required to produce minimal erythema in normal subjects.

At this stage of our knowledge it is no more than a hope that a clear definition of the action spectrum may lead to some understanding of the underlying processes, and ultimately perhaps even of the cause, of polymorphic light eruption.

ACKNOWLEDGMENTS

We wish to thank those clinicians in Aberdeen, Dundee, Edinburgh, Glasgow, Stoke-on-Trent and other centres, who have allowed us to study their patients.

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The construction and development of a grating monochromator and its application to the study of the reaction of the skin to light

L.A.MACKENZIE AND W.FRAIN-BELL

Regional Physics Department, Eastern Regional Hospital Board and
Department of Dermatology, University of Dundee

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SUMMARY

The construction and performance of equipment consisting of a 1600 W xenon arc light source and a grating monochromator are described. Its suitability for use in the investigation of the reaction of the skin to ultraviolet (UV) radiation and to visible light is discussed.

It is a decade or more since Burkhardt (1947), and Beal (1948), and Porter and Magnus in this country, first drew attention to the advantages of a water-prism monochromator and also to the relative disadvantages of a mercury arc compared with a carbon arc or a xenon source for irradiation of the skin; Porter (1954) used a glass-prism; Magnus *et al.* (1959) used a quartz water-prism. In 1967 we described the development of equipment suitable for phototesting (Turnbull, Frain-Bell & Mackenzie, 1967) which consisted of a high-output, water-cooled 6 kW xenon light source with appropriate optical glass filters. Subsequently, its application as a diagnostic instrument in the study of photodermatoses was described (Frain-Bell, Mackenzie & Witham, 1969). Although this type of equipment is of value, the use of optical glass filters to determine the irradiation spectrum is relatively crude and does not allow the controlled selection of a spectral band centred on a particular wavelength. A monochromator with either a prism or a diffraction grating as the dispersing element is essential for this purpose. During the past 3 years we have assembled and constructed equipment consisting of a 1·6 kW xenon light source with a diffraction grating monochromator. The difficulties which have arisen in the development of this equipment and its application to clinical problems are the subject of this report.

DESCRIPTION OF THE APPARATUS

A high pressure xenon arc lamp (Osram Wotan XBO 1600 W) is used with variable d.c. power supplied from a three-phase rectifier unit (Automat Ltd.) (Fig. 1). The protective housing for the lamp (B) is air cooled by convection, the hot air and ozone being discharged by an extraction fan. A fused silica lens (focal length 50 mm, diameter 50 mm) produces an image of the arc on the entrance slit of a Bausch and Lomb 500 mm grating monochromator (Type 33-86-45). The diffraction grating (H) is ruled with 600 lines/mm with a blaze wavelength of 300 nm. The wavelength drum is marked in

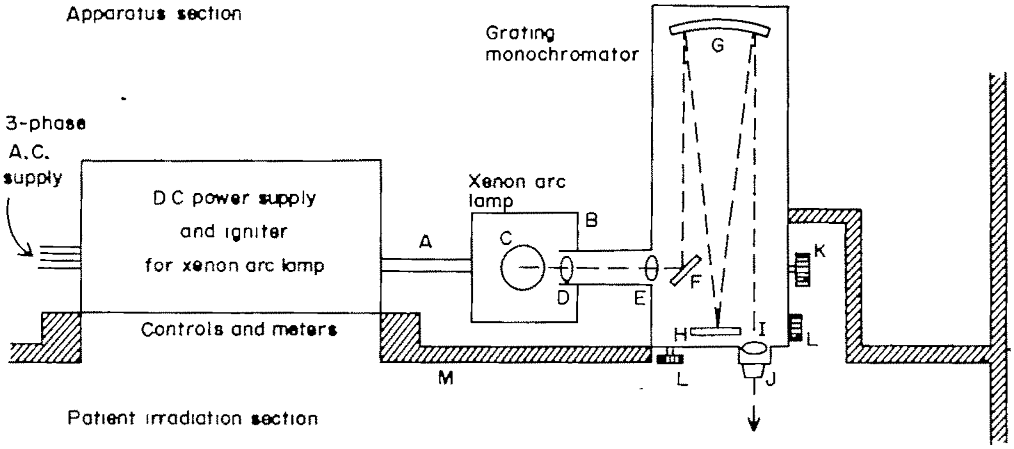

FIGURE 1. Schematic layout of equipment, as seen from above: A, high tension cables; B, protective lamp housing; C, 1600 W xenon arc lamp; D, condenser lens; E, entrance slit and lens; F, plane entrance slit mirror; G, concave collimating mirror; H, diffraction grating; I, exit slit and lens; J, shutter, filter holder and nozzle; K, wavelength control; L, slit width controls; M, heat proof, light-tight partition.

nanometre divisions from 200 to 1400 nm (K). The reciprocal linear dispersion of the monochromator is 3·3 nm/mm, and the entrance and exit slits are adjustable in width up to 12 mm, giving a maximum bandwidth of about 40 nm (L). A fused silica field lens (focal length 200 mm) at the entrance slit, together with the collimating mirror (G) within the monochromator produce an image of the condensing lens aperture near to the diffraction grating. This image does not completely fill the grating (Johns & Rauth, 1965a). A fused silica field lens (focal length 60 mm) at the exit slit (I) together with the collimating mirror produce an image of the diffraction grating, approximately 12 × 12 mm, at about 60 mm in front of the exit slit. An 8 mm diameter irradiation field at this plane is defined by a nozzle. Between the exit slit and the nozzle is an electrically timed shutter and the optical filter compartment (J). The nozzle can be removed from its clamp and replaced by a thermopile detector (Hilger-Schwarz FT4.1). This detector has a 2 mm diameter receiver disc; the spectral response is stated to be uniform over the range 180–1400 nm. The detector response time is 0·04 s and the sensitivity is typically 1·1 mV/mW, i.e. 30 mW cm^{-2} per millivolt. The maximum irradiance limit is 600 mW cm^{-2}. The thermopile e.m.f. is measured directly using a digital voltmeter (Solartron DM1420) with a sensitivity of 2·5 μV. The Hilger-Schwarz thermopiles are liable to damage by electrical fields and rough handling, and should be kept at least 4 feet away from the xenon arc when it is being struck. Several thermopiles, including one with greater sensitivity, should be available and frequent intercomparisons are advisable to check on their constancy.

A light-proof, heat-resistant partition shields the operator and patient (M).

Johns & Rauth (1965a) have detailed the reasons for preferring a diffraction grating rather than a water-prism as the dispersing element for use in the wavelength range 200–300 nm. Briefly, the blazed grating monochromator has constant dispersion, it requires much less routine servicing, it is optically more stable and more efficient, and it produces less stray radiation to contaminate the exit beam. Ease of purchase and of routine operation confirmed the grating monochromator as the choice in our apparatus, particularly for wavelengths greater than 300 nm. Output flux is theoretically proportional to the number of lines/mm ruled on the grating, but the more finely ruled grating has a smaller wavelength range and probably a faster rate of deterioration in use.

The apparatus allows for vertical and lateral adjustments of the arc position, and for independent adjustments of the arc and the condensing lens along the optical axis. These adjustments are essential on commissioning or after a lamp replacement, in order to achieve a uniform output field of maximum irradiance.

Function of the apparatus

In the application of such a monochromator to the study of patients with photodermatoses certain factors have to be considered if reliable results are to be obtained.

The most important aim is to achieve a high incident radiant energy per second per square centimetre of skin. This quantity, 'the irradiance', may be expressed in units of millijoules per second per square centimetre (mJ s^{-1} cm^{-2}), i.e. milliwatts per square centimetre (mW cm^{-2}). The product of the irradiance and the exposure time in seconds, is the incident radiant energy per square centimetre, termed the 'exposure dose'; it is with this quantity that skin response is usually compared. (The reflection, re-emission and absorption of the incident energy are wavelength dependent and probably subject to significant variation from one individual to another.) The irradiance can obviously be increased by concentrating a given radiant flux emerging from the monochromator onto a reduced area of skin. There is evidence that the response for smaller areas depends on the area as well as the exposure dose (Olsen, Sayre & Everett, 1965). A practical lower limit, about 0·5 cm^2 (8 mm diameter circle), is set to the area of irradiated skin because of the observer's difficulty in recording morphological changes in smaller areas of skin. The irradiance decreases as the selected waveband is narrowed, and it follows therefore that greater precision in measuring an action spectrum can only be achieved by increased exposure times. In practice, the irradiance falls very rapidly as the width of the waveband is decreased—approximately in proportion to the square of the bandwidth—and a compromise between precision and overall total irradiation time is unavoidable. The irradiance must be sufficiently high to allow for practicable patient exposure times, not just for a single exposure but for a series of graded exposures at several wavelengths throughout the range of interest.

That fraction of the radiation which reaches the skin and is outside the selected waveband—'the spectral impurity'—must be kept sufficiently low to ensure that skin responses are in fact produced solely by radiation within the selected waveband. The exposure doses required to produce skin responses at different parts of the UV and visible spectrum vary by many orders of magnitude, and impurity radiation from the highly reactive regions of the spectrum must therefore be reduced to negligible amounts. The realization that 100 times the energy is required for narrow band irradiation at 315 nm to produce a minimal erythema response of the skin compared with that required at 300 nm, highlights the importance of spectral impurity. Thus, reliable deductions as to action spectra for erythema or other abnormal skin responses cannot be made if significant spectral impurity is present. It is possible, because of rapid change of reactivity with wavelength, even without significant spectral impurity, that erythematous responses can be produced predominantly by the radiation at the extremity of the chosen bandpass, particularly if the bandwidth is relatively broad. For example, with a central wavelength of 335 nm and a half bandwidth of 30 nm (equal entrance and exit slit widths of 9 mm), theoretically about 1·5% of the total radiation is of wavelengths 305–310 nm. This small percentage of highly reactive radiation may be sufficient to produce a skin response which, being attributed to the central wavelength 335 nm, produces a distortion in the derived action spectrum. In general, such distortion is inevitable because of the use of finite bandwidths, but its degree can be controlled to some extent by judicious choice of the bandwidth used for a particular investigation.

The exit beam from the monochromator includes wanted radiation in the selected waveband together with unwanted radiation outside this waveband. The unwanted radiation is of two distinct types; firstly, stray radiation scattered towards the exit slit from inside the monochromator and

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secondly, the second or higher order spectral radiation inherently produced by the diffraction grating. Stray radiation, which is produced by scattering and reflection by the internal structures and optical surfaces of the monochromator, forms a background continuous spectrum of all wavelengths present at the entrance slit. The total energy contribution from stray radiation depends upon monochromator design and operation and upon the condition of the optical surfaces. Stray radiation can be reduced by restricting the range of wavelengths entering the monochromator: for example by interposing a selective filter or mirror between the arc lamp and the entrance slit, or by placing a second grating monochromator or a pre-prism before the existing monochromator, thus forming a double monochromator. In practice this is inevitably accompanied by some reduction in the wanted radiation level. The method which we have adopted is to use an optical glass filter 2·5 mm thick (Schott) between the exit slit and the skin, with no wavelength restriction at the entrance slit. One of a range of Schott cut-off filters is chosen so that the filter transmission at the short wavelength extremity of the monochromator waveband is about 50%, but with a rapid fall in transmission at shorter wavelengths than this. Filters which fluoresce strongly are excluded. Suitable filters for use with central wavelengths above 335 nm are listed in Table 1. These filters absorb stray radiation only on the short wavelength side of the selected waveband. Their use virtually eliminates the second and higher order spectral radiation which appears for central wavelengths above about 440 nm.

TABLE 1. Optical glass filters (Schott) suitable for use with central wavelengths above 335 nm

Central wavelength (nm)	Filter
Below 335	None
335–374	WG305
375–409	WG345
400–444	GG375
445–499	GG420
500–574	GG475
545–575	OG515
576–619	OG570
620–699	OG590
Above 700	RG665

Although numerical values for stray radiation levels have been quoted by those describing monochromator systems, there appears to be no generally accepted definition of this quantity, or a convention on how it should be measured and expressed. The main reason for this unsatisfactory situation is undoubtedly the technical problems associated with the measurement of stray radiation. Johns & Rauth (1965b) have described some of these problems, encountered under laboratory conditions, in a limited spectral range using a high pressure mercury arc. The measurement of stray radiation is associated with measurement of the shape of the spectral band emerging from the collimator. Johns & Rauth (1965a) describe theoretical bandpass shapes and Berger (Urbach, 1969) discussed both bandwidth and stray radiation, emphasizing the importance of these quantities, but giving insufficient detail of measurement methods and interpretation. Relatively crude methods of estimating stray radiation from measurements with and without optical filters in the beam are imprecise. Sufficient sensitivity and spectral resolution in these measurements can only be achieved by using a spectroradiometer. This entails incorporating an additional monochromator of known stable characteristics, carefully coupled to the output of the main monochromator system, and a detector of known spectral sensitivity, capable of handling a very wide range of radiation levels. A spectroradiometer of this type may well be comparable in expense with the main monochromator system, but this may be justified by its use in periodic checks of spectral band shapes and of stray radiation levels, as the main system deteriorates. We have made relatively crude estimates of the stray radiation, from measurements of the output, with and without a filter cutting off the short wavelength extremity of the waveband. These suggest that stray radiation of shorter wavelengths does not constitute more than 1% of the total energy output.

Performance

The efficiency of a grating monochromator, that is the ratio, at a particular wavelength, of the output radiant power to the input radiant power, depends upon the wavelength (Fig. 2) and is markedly influenced by the 'blaze' wavelength of the grating. The output of the xenon arc lamp also is wavelength dependent. It follows, therefore, that the output of a xenon arc/grating monochromator combination is determined by the product of these two spectral distributions and is further modified by any wavelength dependent factors associated with the entrance and exit optical systems. All these various factors can alter as the equipment deteriorates with usage. It is not practicable to make routine checks of each of the wavelength dependent factors separately, but routine measurements of the output irradiance throughout the wavelength range can be made at fixed conditions of lamp power, slit width and height, etc. (Fig. 3), and used to assess deterioration.

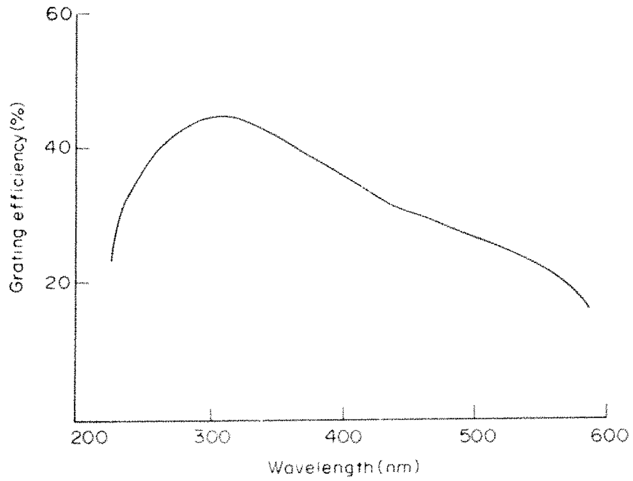

FIGURE 2. A spectral efficiency curve for a 600 line/mm grating, blaze wavelength 300 nm, based on manufacturer's data.

With experience, skin irradiation schedules have been prepared for the various abnormal photosensitivity states, specifying the exposure doses, central wavelengths and bandwidths. The most common choice of entrance and exit slit widths, 9 mm, gives a half-maximum bandwidth of about 30 nm. For more sensitive skins and more reactive parts of the spectrum, the output irradiance may be reduced in several ways, whilst maintaining the same central wavelength for the waveband.

(1) The entrance and exit slit widths can be reduced but kept equal. The bandwidth is thereby reduced but the spectral distribution within the band remains triangular in shape.

(2) The entrance slit width can be reduced leaving the exit slit unchanged. The half-maximum

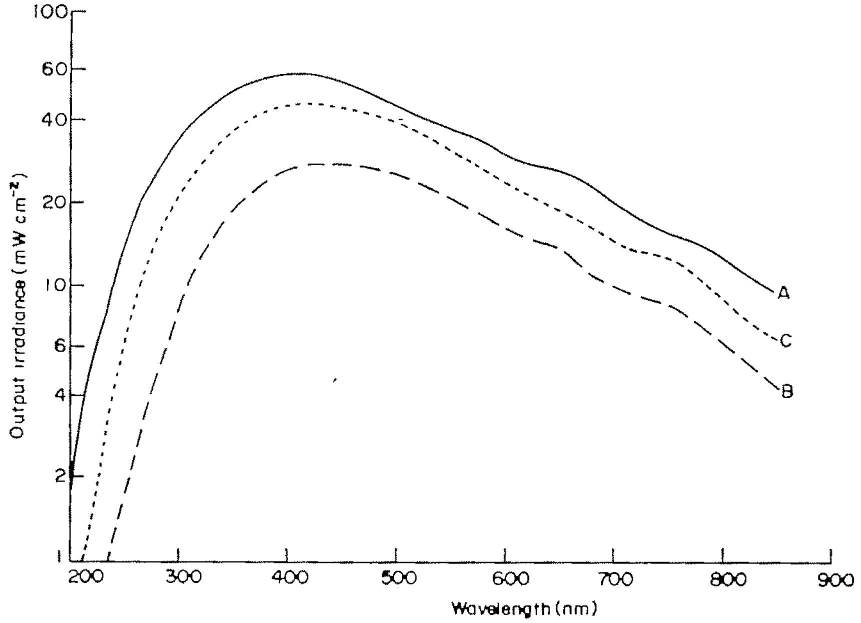

FIGURE 3. Variation of monochromator output irradiance with wavelength (16.5 nm half maximum bandwidth). Entrance and exit slit widths 5 mm, slit height 20 mm. ———, initial condition; – – – – –, after 670 h usage (monochromator mirrors as in Fig. 6); ...., partial recovery of output on renewal of mirrors.

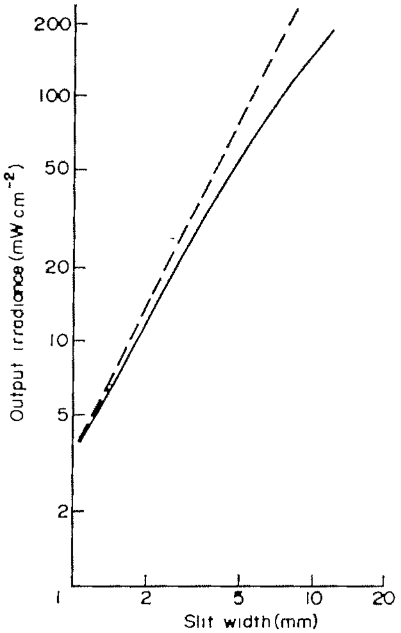

FIGURE 4. The effect of slit width on output irradiance. Equal entrance and exit slits. Half maximum bandwidth (nm) = 3·3 × slit width (mm). ———, measured values; – – – –, theoretical values.

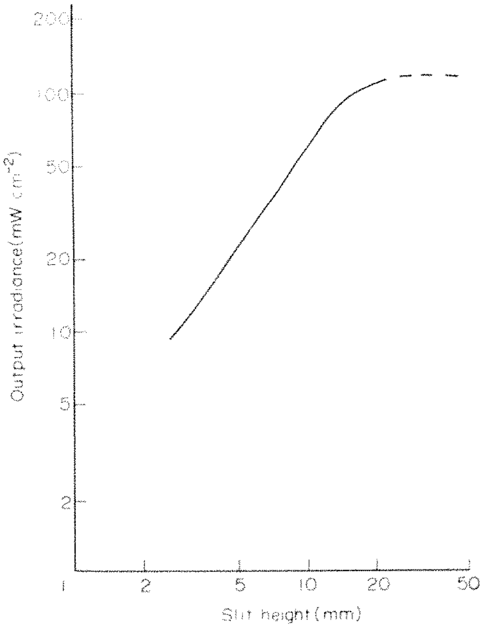

FIGURE 5. The effect of slit height on output irradiance. Entrance and exit slit widths constant at 9 mm. Central wavelength 400 nm.

bandwidth then remains unchanged but the spectral distribution becomes more rectangular in shape.

(3) The entrance or exit slit heights, or both, can be reduced. This maintains both the bandwidths and the band shape. The effect of changes in slit width and height on the output irradiance are shown in Figs. 4 and 5. These graphs, which are used for predicting slit settings for new irradiation conditions, also indicate the rapid fall in irradiance resulting from reductions in bandwidth.

Optimum uniformity of irradiance within the 8 mm diameter irradiation field is achieved by adjustments to the entrance optics and the arc position. Measurements using a thermopile with 3 mm diameter receiver suggest that the irradiance is uniform to within ± 10%. Visually the field appears spectrally homogeneous, although at several centimetres from the plane of the exit nozzle, there is noticeable spreading of the beam and spatial separation of the wavelength components within the waveband. The output irradiance falls slowly at a rate of about 3%/mm with increasing axial distance from the irradiation plane, and so the positioning of the skin relative to the entrance nozzle is not critical.

Deterioration

Routine measurements of output irradiance at various central wavelengths showed a rapid fall in output of about 30%/100 h, during the first 150 h of usage, affecting the wavelengths below about 300 nm more than those above. Subsequently the deterioration was much less rapid. At 350 h, the lamp power was eventually increased in order to restore output. This was followed by a second rapid fall in output and subsequent less rapid fall after about 150 h. Again the shortest wavelengths were the most affected. It was thought at this stage that the major factor involved was a decline in the output of the xenon arc lamp. However, after 670 h usage, the monochromator covers were removed and gross deterioration of the mirror surfaces was found (Fig. 6).

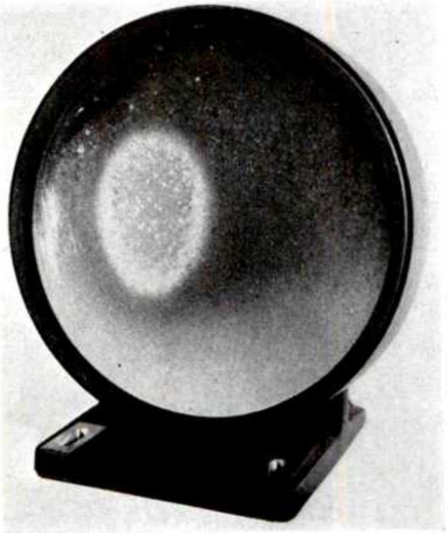

FIGURE 6. Monochromator collimating mirror. Deterioriation of mirror surface where entrance beam impinges, after 670 h.

The plane entrance slit mirror and the concave collimating mirror were both affected where the incoming beam had been incident. The mirrors were renewed and a substantial but incomplete recovery of output was achieved (Fig. 3). This suggested that deterioration in other components which was more marked at the shorter wavelengths had also taken place. Damage to the mirror surfaces is probably due to the combined effect of ozone and heat, and methods to minimize these factors are being studied. Meanwhile, the mirror surfaces are inspected visually at intervals of 200 h of usage and a spare set of mirrors is available for replacement if required.

Although the average lifetime of the xenon arc lamp is quoted as 2500 h, it is likely that reduction in output in the wavelength range 200–400 nm will be more marked than for longer wavelengths, during the lifetime of the lamp. The accompanying reduction in ozone production may account for the rapid and slow phases in irradiance deterioration previously mentioned.

Deterioration in the grating performance with use is uncertain and difficult to check routinely. The deterioration of the grating and mirror surfaces will result not only in progressive reduction in output irradiance but will inevitably increase the amount of scattered radiation within the monochromator. How much this affects the spectral purity of the output beam is of obvious importance and requires further study.

DISCUSSION

In 1965 Sayre and his co-workers described the construction and use of a high intensity UV light monochromator consisting of a 2·5 kW xenon source and a Bausch and Lomb grating monochromator (33-86-25) (Sayre *et al.*, 1965). The light was passed through a water-cooled UV transmitting filter coloured system prior to entering the monochromator, thus reducing heating of the monochromator components by energy, particularly infra-red, from outside the desired spectral band. By selection of filters with different transmission properties, this water-cooled system could be modified for use in other portions of the light spectrum. The irradiance at a central wavelength of 310 nm and bandwidth of 10 nm was reported to be 7 mW cm^{-2}, which produced a minimal erythema with as little as 1 s exposure. In 1969 Sayre reported that the stray light found with this equipment was not more than 1%.

In 1967, Knox and his co-workers described the design of a high intensity monochromator consisting of three independent monochromator units (Bausch and Lomb 33-86-25), the output from each unit being superimposed on a test site of area approximately 1 cm^2 (Knox *et al.*, 1967). The light source for each monochromator was a 150 W xenon arc lamp (Bausch and Lomb 33-86-20-01). Alternative diffraction gratings were available for each of the three monochromators, one for UV and one for use in the visible parts of the spectrum. The construction was such that during irradiation it was also possible to divert the beam temporarily to a detector and so record the energy output on a chart. The spectral purity of the output beam was stated to be 99%. Selective filters were used to minimize the second order spectrum and to reduce stray light. They stated that the scattered radiation in the monochromator was only of significance when using the UV gratings. Filters with cut-off points at approximately 320 and 360 nm respectively were used when it was necessary to eliminate second order effects with the visible light grating. The exposure time required to give a minimal erythema dose to the untanned human skin of the abdomen, using wavelengths between 250 and 300 nm, was usually 15–25 s, but these times were as low as 4–6 s at 300 nm using a 5 nm bandwidth.

In a symposium on the biological effects of UV irradiation, Berger *et al.* (1969) emphasized that misleading results could be caused by the relatively high stray light levels of single monochromators. The stray light level can be considerably reduced by passing the light from one monochromator through a second monochromator, although this inevitably results in a reduction in output in the selected waveband. This can be offset by an increase in exposure time or bandwidth, or by using a lamp of greater power. For example, if a monochromator with 0·1% stray light is combined with a second similar monochromator to form a double monochromator, the stray light level would be reduced to 0·1 × 0·1% = 0·0001%. If the overall efficiency of the single monochromator is 40% at a particular wavelength, then for a double monochromator it is 40% × 40% = 16%. Thus, a reduction of stray light by a factor of 1000 is accompanied by a reduction in wanted output by a factor of 2·5. Berger in 1967 used a double grating monochromator and an Osram Wotan XBO 2500 W xenon arc lamp (Berger, Urbach & Davis, 1967). He measured stray light of all wavelengths more than 2·5 bandwidths away from the central wavelength, but did not specify the bandwidth of the measuring apparatus or the radiation source used for the measurements. The following stray light level intensities were reported: less than 0·04% of the primary peak intensity at the first exit slit and less than 0·0001% at the second exit slit. More recently, Cripps & Ramsay (1970) have described the double monochromator for which they found that a 2·5 kW xenon mercury lamp gave an adequate output in the UV range. They chose a double monochromator consisting of a prism monochromator followed by a grating monochromator (Johns & Rauth, 1965a) since they considered that a prism would be less subject than a grating to damage by heat. A quartz window water bath and filter holder was incorporated between the lamp and the first entrance slit. Stray light intensities 2·5 bandwidths from the primary peak radiation of an isolated mercury line were reported as less than 1% of the primary peak intensity at the first exit slit and less than 0·05% at the second exit slit.

Table 2 attempts to summarize the performance data for the various systems described in the literature. Although direct comparisons are almost impossible because of differences in lamp power, monochromator type, output field size and bandwidth, the table does indicate the effects of these differences.

The use of the monochromator in the study of reactions of the skin to light

The monochromator, be it of grating or prism type, is the equipment of choice for action spectroscopy

TABLE 2. Summary of published monochromator performance data

Authors	Monochromator type	Lamp power and type	Output field size (cm²)	Bandwidth (nm)	Output irradiance at central wavelength (mW cm⁻²)							
					200 nm	250 nm	300 nm	350 nm	400 nm	450 nm	500 nm	550 nm
Sayre *et al.* (1965)	Single grating	2·5 kW Xenon		10	—	0·5	7·0	10·8	3·1	—	—	—
Knox *et al.* (1967)	Three single gratings	Three 150 W Xenon	1·0	5	0·025	0·09	0·65	1·2	—	2·6	—	2·1
Berger *et al.* (1967)	Double, two gratings	2·5 kW Xenon	0·28	3·5	—	0·25 (254 nm)	2	2·5 (320 nm)	—	—	—	—
Cripps & Ramsay (1970)	Double, one prism and one grating	0·9 kW Xenon	0·16	4	—	—	0·091	0·296	0·432	—	—	—
		1·6 kW Xenon			—	—	0·189	0·590	0·908	—	—	—
		2·5 kW Xenon			—	0·0227	0·408	1·21	1·81	—	—	—
		1·0 kW Xenon–Mercury			—	0·0530	0·726	0·113	0·182	—	—	—
		2·5 kW Xenon–Mercury			—	0·0234	2·19	0·386	0·680	—	—	—
Mackenzie & Frain-Bell (1973)	Single grating	1·6 kW Xenon	0·50	6·6	—	2·7	6·8	8·7	9·3	8·9	6·8	5·4

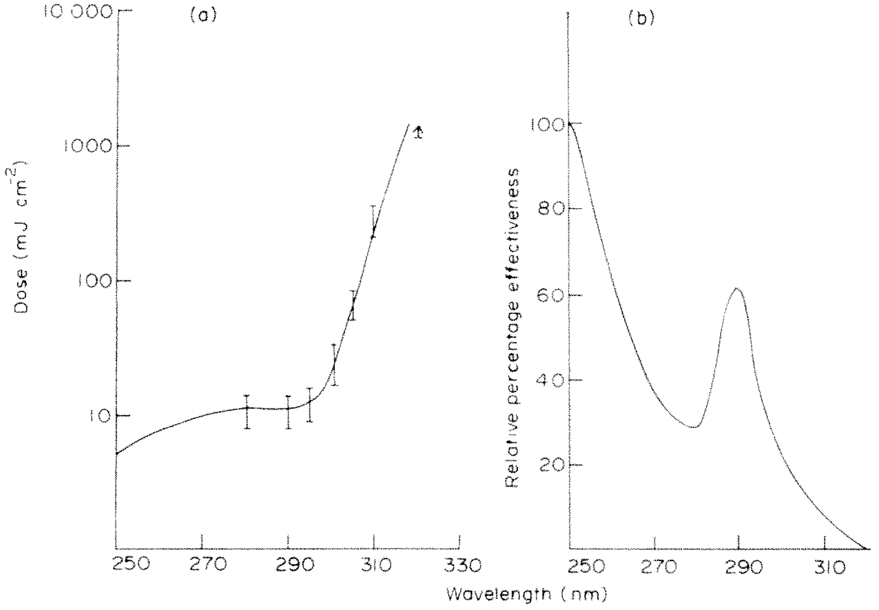

FIGURE 7. Erythema action spectrum in normal subjects. (a) Semi-logarithmic scale based on fifteen subjects showing the erythema action spectrum in normals. The continuous line represents the mean and the bars the standard deviation of the mean. The arrow at 320 nm denotes that the MED was not reached. (b) Percentage effectiveness plot for each wavelength—based on seven subjects. (The MED was taken as the lowest dose regardless of the time of appearance, i.e. whether readings were taken at 7 or 24 h.)

studies of reactions of the skin to light, although the higher output (6 kW) xenon arc cut-off filter system has its uses (Wiskemann & Wulf, 1959; Turnbull *et al.*, 1967). Fig. 7 shows the action spectrum obtained for erythema in normal subjects. It has been shown that this will vary with the bandwidth used and with the interval of time since irradiation, i.e. whether readings are carried out at 7 or 18 or 24 h (Johnson, Herd & Mackenzie, 1972). The figures obtained from the energy values necessary for minimal erythema throughout the UV spectra are given in Table 3. The study of action spectra in the various dermatoses often necessitates irradiation of multiple skin sites which may be as many as 100 or more in the one patient, so that the smaller the individual site is, the better. However, two important aspects of site size must be considered: the first being that more energy per unit area is required and, therefore, an increase in irradiation time to produce the same response in a small area of skin as compared with the larger one (Olsen *et al.*, 1965); and secondly in small areas of skin it is often difficult to recognize abnormal morphological changes other than simple erythema. There is

TABLE 3. Mean MED (250–320 nm) on back skin of twelve normal subjects. The MED was the lowest dose producing a reaction regardless of the time of its appearance.

Central wavelength (nm) (Half bandwidth 5 nm)	250	260	270	280	290	295	300	305	310	320
MED (mJ cm^{-2})	5·6	5·6	8·2	12	10	15	22	68	270	>1200

therefore a limit to the reduction in the area of skin exposed to each irradiation dose. Considering all the relevant factors, an 8 mm diameter field was chosen.

In order to simplify the interpretation of results, exposure times were adjusted according to the measured output irradiance so that at a particular wavelength and bandwidth a group of graded exposure doses are delivered. These doses are always chosen from the appropriate portion of a fixed series of doses increasing in 20% steps, viz. 10, 12, 15, 18, 22 . . ., etc. mJ cm^{-2}.

We believe that all assessment of skin reactions should be made as far as possible by the same observer working under identical lighting conditions and with the patient seated on a high stool in order to avoid variations in erythema, which result if the readings are taken with the subject lying down (Berger, Urbach & Davies, 1967).

In assessing the skin change following irradiation the observer is concerned with the degree of erythema as well as with other morphological changes. The response of the skin was graded as follows: 0, no response; ½, doubtful erythema; 1, minimal but definite erythema; 2, marked erythema; 3, erythema plus oedema. With this method of grading we have found that the human eye appears to be as effective in such assessment as forms of instrumentation such as an EEL reflectometer. Observations of the resultant erythema, etc. were performed immediately following irradiation and at 7–8 h, at 24 and 48 h, and later if necessary. It is important that this schedule of times is adhered to since we have found that erythema responses due to radiation of wavelengths 290–305 nm may be maximal at 8 h, and fading by 24 h, whereas those due to longer wavelengths are maximal at 24 h, but have not developed at 8 h (Herd & Johnson, 1972). In addition, abnormal skin changes such as erythema may be present at 7–8 h, but have cleared by 18 or 24 h. The recording of immediate responses is important, since immediate pigment darkening, urticaria, and short-term erythema may be missed if recordings are made only at 7, 24, and 48 h. Similarly, delayed reactions may be missed if readings are only made at 7 and 24 h, e.g. in xeroderma pigmentosum, where the erythema resulting from short UV wavelengths may not be apparent until after the first 24 h.

Because of the necessity to position the patient close to the monochromator exit slit so as to obtain maximum irradiance levels and thus reduction of exposure time, physical problems can arise from pressure constriction of the skin by the small output nozzle, which was found to sink easily into the tissues of the skin of the back in normal well-covered individuals. It was possible to modify this effect although the problem has not been entirely solved. It does, however, raise the question of the significance of petechiae in the skin following irradiation, which may sometimes be due to a non-specific pressure effect rather than a photobiological action.

It must be recognized that the response of the skin to UV irradiation will vary with anatomical site and even in the same individual at different times. We have noticed that minimal erythema doses may depend on whether or not the subject takes a bath prior to irradiation, and whether there has been prior topical therapy, systemic drug administration, change in ambient temperature, and a number of other variables. We have recently shown (Sturrock, Herd & Frain-Bell, 1972) that although there is an undoubted variation in response of the skin of the back depending on the area irradiated, e.g. between the centre and the periphery and between the top and bottom, this variation is on the average within 20%, this being relatively unimportant provided the system of graded doses (20% steps) referred to above is used.

The investigator must appreciate at all times the limitations of the equipment and the technique, since exposure to sunlight will result in exposure of the skin to a 'solar spectruma'—state of affairs which may differ in its effect from that obtained from localized irradiation of a small skin site with a chosen narrow bandwidth. The development of the type of equipment described is a continuing process, leading in time to what one hopes will be the production of radiation of acceptable spectral purity. However, the instrument described in this report has proved to be suitable for the study of a variety of 'photosensitivity' responses, such as are found in actinic reticuloid, xeroderma pigmentosum, porphyria and summer prurigo; in polymorphic light eruption (Frain-Bell *et al.*, 1972); in lymphocytoma (Frain-Bell & Magnus, 1971); in atopic children (Frain-Bell & Scatchard, 1971); in the assessment of light protecting agents (MacLeod & Frain-Bell, 1971); in photocontact dermatitis (Gardiner *et al.*, 1971); and in those responses resulting from systemic administration of drugs and as a result of exposure to light in conditions such as psoriasis, lupus erythematosus, etc.

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The action of solvents on the ultrastructural appearance of keratohyalin and the horny layer in guinea-pig back epidermis

R.I.C.SPEARMAN AND J.A.HARDY

Department of Dermatology, University College Hospital Medical School, London, W.C.1

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SUMMARY

Various solvents were used to remove osmium-reactive phospholipids and free fats from guinea-pig back epidermis before post-fixation in osmium. After extraction, the osmium stained material which remained was probably due to proteins with reactive amino acids. After brief fixation in glutaraldehyde, followed by immersion of the tissue in pyridine, a phospholipid solvent, followed by osmium post-fixation, the unkeratinized epidermal cells were practically unstained. Removal of phospholipid from the keratohyalin granules revealed them as aggregates of smaller particles, probably the protein component. The thickened plasma membranes of the horny cells were darkly stained in osmium. Removal of material from the interiors of these cells left empty spaces between the meshwork of filaments, indicating absence of matrix keratin. The contrast between the darkly stained keratinized cell membranes and filaments, and unstained unkeratinized cells was marked after lipid extraction.

Since the publication of the first good electron micrographs of guinea-pig and human epidermis by Brody (1959, 1962, 1964), a gulf has arisen between findings at the light microscopical histochemical level, and those at the ultrastructural level. By light microscopy the horny layer in both paraffin embedded human arm and guinea-pig back skin is subdivisible into a thin compact phospholipid rich conjunctum region adjacent to the granular layer, and above it a thicker disjunctum region composed of swollen empty cells arranged in tiers without discernible intercellular spaces (Szakall, 1958; Spearman & Riley, 1967; Spearman, 1970). Some intracellular material is retained in these cells in frozen sections untreated with solvent (Christophers, 1971). In palmar and plantar horny cells, eosin stainable cytoplasm occurs even in paraffin sections, and in parakeratosis there are also pyknotic remnants of nuclei (Jarrett & Spearman, 1964).

In comparison, electron micrographs of osmium fixed human and guinea-pig hairy skin show the epidermal horny cells much more flattened, and the spaces between them, above and below, are wider than between the prickle cells. The horny cell plasma membranes are characteristically thickened, and the cell interiors contain a mass of filaments apparently embedded in an amorphous, weakly electron dense material, which is largely lost by the time the cells are desquamated (Breathnach, 1971). The extreme rarity of organelle remnants in this type of horny layer is evidence of the characteristically extensive autolysis which occurs during horny layer formation in all hairy skin (Jarrett & Spearman, 1964; Lavker & Matoltsy, 1970).

Osmium, the best all-round fixative for electron microscopy, reacts with certain amino acids, which include cystine, cysteine, histidine, and tryptophan, and also with unsaturated fatty acids in free fats and phospholipids (Bahr, 1954). In consequence, soluble reactive substances are precipitated (Wolman, 1957), and these cannot then be distinguished from amorphous structural elements in the horny layer.

That much of the amorphous material observed in the horny cells in electron micrographs is due to precipitation by osmium of normally soluble unsaturated lipids was shown previously by experiments with solvents at the light microscopic level (Spearman & Riley, 1967; Spearman, 1970).

In the present investigation, various lipid solvents were applied to guinea-pig back skin before post-fixation and staining in osmium, in order to determine effects of lipid extraction on the ultrastructural appearance of the epidermis, and in particular on keratohyalin and the keratinized cells. Of these methods, extraction in petroleum ether followed by ethanol and water has been widely used to remove sebum from hair for electron microscopy (Johnson & Speakman, 1965).

In life, the horny layer is continuously exposed to the leaching effect of water in transpiration, but this fails to remove osmophilic material from the epidermis. Loss of degraded cell constituents from horny cells about to be desquamated is, however, probably due to extraction in this way.

MATERIALS AND METHODS

Guinea-pig back epidermis was examined in all procedures. Contralateral sites were used for extraction in solvents and as untreated controls. Hair was clipped short with scissors and the skin was not shaved or chemically depilated.

Extraction procedures

Petroleum ether, absolute ethanol, and distilled water. Various procedures were used, but the following method was finally adopted. A piece of skin was excised from a recently killed guinea-pig and then spread out over the opening of a glass vial filled with solvent. It was held in place by a rubber band and the vial inverted so that the fluid was in contact with the horny layer. The exposed dermal side was covered with a swab moistened in normal saline and placed in a beaker containing crushed ice. The beaker was then placed in a cold room at +4°C.

The vial was first filled with petroleum ether and left for 1 h; it was then replaced by mixture of equal parts of ethanol and petroleum ether for 10 min, followed by absolute ethanol for 1 h, and then by distilled water for 10 min. An area of epidermis approximately 1 mm diameter, was dissected from the centre of the site and fixed in 3% glutaraldehyde solution in pH 7·3 phosphate buffer.

Other specimens were placed only in petroleum ether and water, and in absolute ethanol and water, respectively. Untreated skin was placed in fixative at the start of the experiment.

After fixation for 24 h in glutaraldehyde, the specimens were washed for 48 h in buffer solution, and then post-fixed for 2 h in a 2% solution of osmium tetroxide in pH 7·3 buffer. After washing in buffer solution and dehydration in ethanols, the specimens were embedded in Araldite.

Pyridine. Approximately 1 mm diameter pieces of skin were removed from a recently killed guinea-pig and fixed immediately in buffered glutaraldehyde for 6 h. If fixation is prolonged, bound lipids cannot be extracted in pyridine (Jarrett *et al.*, 1965). The tissue was washed in the buffer solution for 12 h, and then immersed in pyridine for 4 h. Afterwards it was washed in buffer, with one change, for 12 h. All trace of pyridine must be removed, as it reacts with osmium. The tissue was then post-fixed in osmium for 2 h, washed, dehydrated, and embedded in Araldite.

Control untreated tissue was kept in buffer solution while the other specimens were in solvent. This failed to remove osmium stainable material. Some tissue was fixed in glutaraldehyde and either extracted or not extracted in pyridine, but not treated with osmium before embedding.

Chloroform. The procedure was as for pyridine, with chloroform replacing pyridine.

In all these procedures fixation and washing was carried out at +4°C. Extraction in pyridine and in chloroform was at room temperature.

Examination of tissue

Araldite blocks were cut on a Reichert OmU2 ultramicrotome with a diamond knife, and sections were mounted on copper grids for examination in either a Siemens Elmiskop II or AEI 6MB transmission electron microscope, at magnifications of × 5000 to × 100,000, at 60 kV.

Some sections in each group were counterstained on the grids in uranyl acetate to increase contrast of nuclei in unkeratinized cells.

RESULTS

Petroleum ether, absolute ethanol and water

The horny layer. Cells throughout the depth of the horny layer appeared swollen and more uniform in size than in control unextracted osmium fixed tissue. Intercellular spaces were narrow in contrast to the wide spaces normally seen in EM preparations of horny layer. Some osmium reactive material remained in the intercellular spaces after extraction, and the plaques between opposing hemidesmosomes were not removed. The basal zone of the horny layer—the phospholipid-rich conjunctum region in light microscopy (Fig. 1)—normally contains much more osmium-stainable amorphous material in the interiors of the cells, than in the intermediate and superficial zones. This amorphous material was readily removed by extraction, leaving behind only a mass of short, randomly orientated filaments with nothing in the interstices except embedding resin. These filaments appeared more widely spaced than in unextracted cells. The thickened plasma membranes were darkly stained, and more sharply

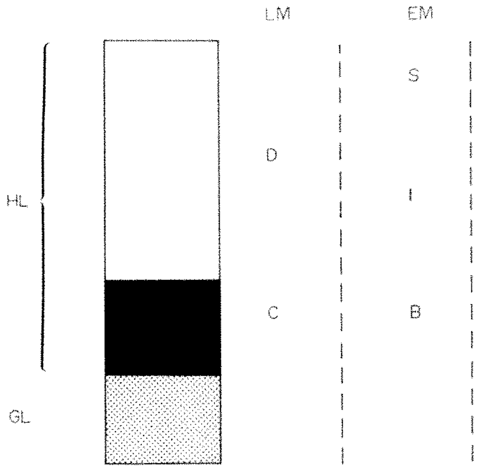

FIGURE 1. Topography of the stratum corneum in hairy skin by light microscopy, LM, and electron microscopy, EM. Horny layer, HL; disjunctum zone, D, without phospholipid. Conjunctum zone, C, contains phospholipid. This is the basal region, B, in EM. The intermediate zone, I, and superficial zone, S, correspond with the disjunctum zone. Granular layer, GL.

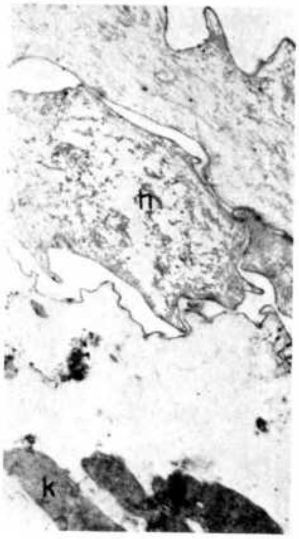

FIGURE 2. Guinea-pig back epidermis to show topography after extraction in pyridine. Keratohyalin in granular layer, k; basal horny cells, h. (× 7500).

defined than in unextracted horny layer. Beneath the plasma membrane there was only a thin layer of more weakly stained cytoplasm.

Absolute ethanol in the absence of petroleum ether was as effective as the combined procedure in removing amorphous material (Fig. 4). Petroleum ether alone was less effective in the basal zone, but material was readily removed from the intermediate zone (Fig. 3).

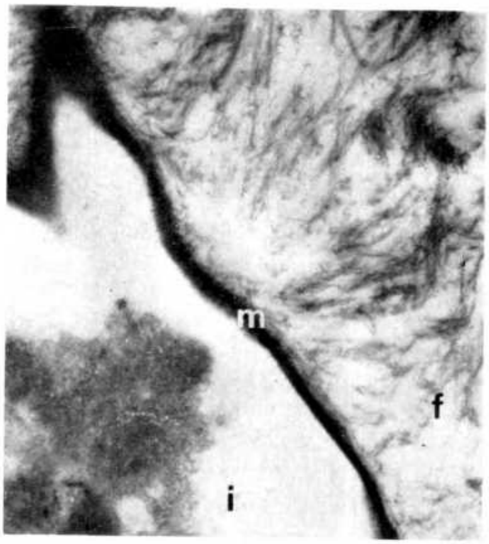

FIGURE 3. Guinea-pig back epidermal horny layer. Amorphous material in intermediate zone cell was extracted in petroleum ether before fixation in osmium. Filaments, f; darkly stained plasma membrane, m; material in intercellular space, i. (× 55,000).

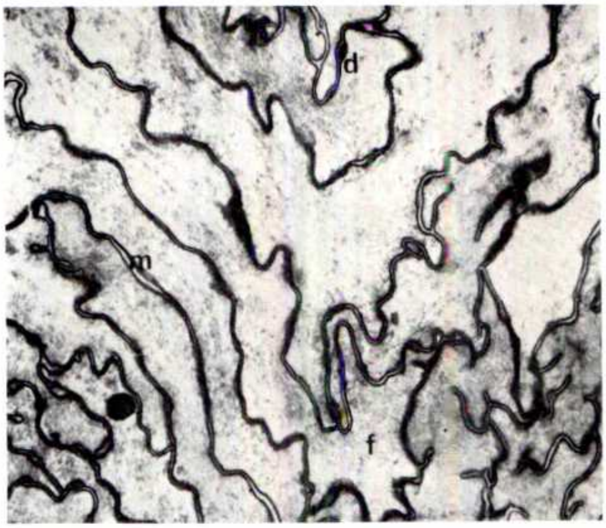

FIGURE 4. Guinea-pig back epidermal horny layer. Amorphous material in the cells has been dissolved out in absolute ethanol, leaving only a mass of short filaments, f. The plasma membranes are darkly stained in osmium and are in close opposition with only narrow intercellular spaces, m. Desmosome, d. The lower half of the field is in the basal zone, and the upper half in the intermediate zone of the horny layer (× 8000).

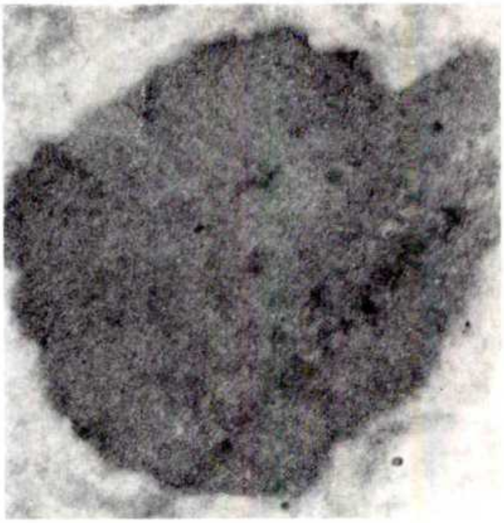

FIGURE 5. Keratohyalin granule in guinea-pig back epidermis not treated with solvents. Fixed in glutaraldehyde and osmium. It has a faintly speckled appearance (× 30,000).

Cells deep to the horny layer probably did not receive much solvent by surface application and will not be considered.

Pyridine

Unkeratinized epidermal cells. After extraction, the basal and prickle cell layers were very poorly stained in osmium, with the plasma membranes ill-defined.

The granular layer. Plasma membranes and cytoplasm were as poorly stained, as in the basal and prickle cell layers, and the only electron-dense material was keratohyalin (Fig. 2). In control unextracted skin fixed in osmium, keratohyalin granules varied in size, but tended to occur in larger lumps than in human hairy skin. They were darkly stained in osmium with a barely perceptible speckled appearance (Fig. 5).

After pyridine extraction of phospholipid and osmium post-fixation, keratohyalin granules were more clearly shown to be aggregates of much smaller stained particles (Fig. 6). There was no sign of filaments in the extracted keratohyalin or of fibres derived from these aggregates, as has been suggested may occur. Unassociated, poorly stained filaments occurred in the cytoplasm.

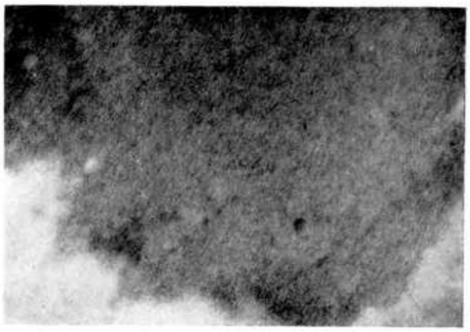

FIGURE 6. Keratohyalin in guinea-pig back epidermis. Fixed in glutaraldehyde, followed by pyridine extraction and osmium post-fixation. Some amorphous material has been removed from between the small particles in the granule (× 30,000).

The horny layer. The EM appearance broadly resembled the effects of absolute ethanol and petroleum ether, in that amorphous material was removed from the cell interiors and the cells were swollen even in the basal zone (Figs. 2, 8 and 9). The thickened plasma membranes were darkly stained and well defined. The internal filaments were more electron dense than in the granular cells.

The appearance of glutaraldehyde fixed epidermis untreated with osmium was broadly similar to extracted and osmium-fixed tissue, with prominent horny cell plasma membranes and keratohyalin.

Chloroform

Unkeratinized epidermal cells. Osmium-reactive material was removed from the unkeratinized epidermal cells as in pyridine extraction, and the membranes were ill-defined.

The granular layer. Amorphous interstitial material was removed from between the small particles in keratohyalin granules, and the particles appeared more dispersed than after pyridine, which suggested better extraction in chloroform (Fig. 7).

The horny layer. The thickened plasma membranes were darkly stained and well defined, but

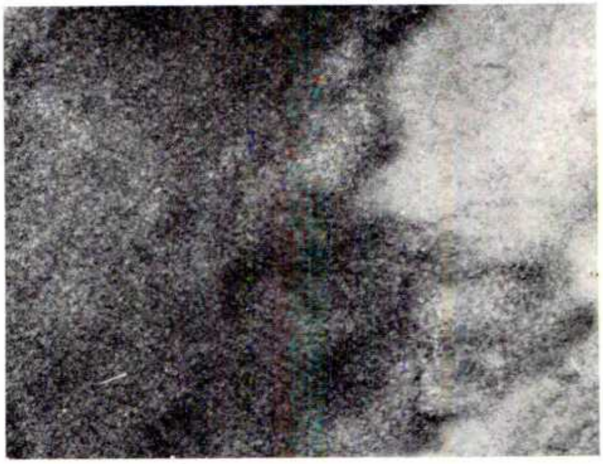

FIGURE 7. Keratohyalin granule in guinea-pig back epidermis after chloroform extraction. More material has been removed from between the small particles than by pyridine (× 40,000).

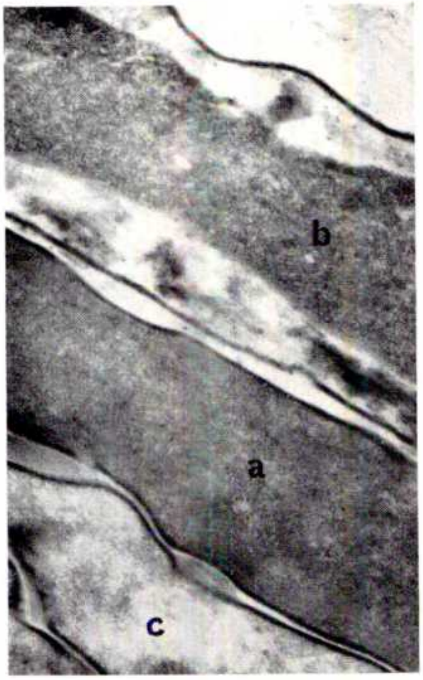

FIGURE 8. Guinea-pig back horny layer basal zone. Stages in the extraction of amorphous material from the cells by pyridine before osmium post-fixation. Little material removed in cell a, partial extraction has occurred in b, and complete removal of phospholipid has occurred in c. Note the prominent plasma membranes (× 30,000).

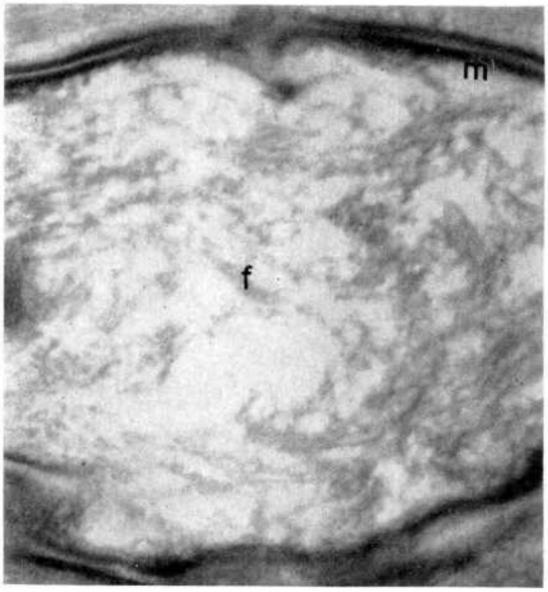

FIGURE 9. Guinea-pig back horny layer intermediate zone. Pyridine extraction and osmium post-fixation. Dark plasma membranes, m, with lining cytoplasm. The cell interior contains filaments, f, but no matrix keratin (× 50,000).

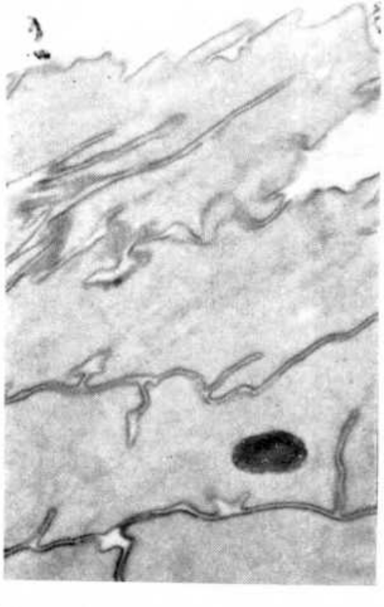

FIGURE 10. Guinea-pig back horny layer superficial zone. Glutaraldehyde fixation, chloroform extraction and osmium post-fixation. Little amorphous material has been extracted from the cells by this non-polar solvent, but the intercellular spaces are narrow and the plasma membranes are prominent. Note the nuclear remnant (× 15,000).

removal of material from inside the horny cells was incomplete (Fig. 10) in contrast to the action of chloroform on keratohyalin.

DISCUSSION

Removal of material normally stained by osmium by extraction in lipid solvents shows that most of the staining by osmium seen in electron micrographs is caused by free or weakly-bound lipids with unsaturated groups; much less is due to proteins. Pyridine, a phospholipid solvent (Pearse, 1970), removed material normally stainable in osmium, probably phospholipid, from the unkeratinized epidermal cell plasma membranes and cytoplasm, from between the small particles in aggregates of keratohyalin, and from the interiors of the horny cells, where the amorphous material between the electron dense filaments was lost. The subparticulate nature of keratohyalin, perceptible as a faintly speckled appearance in unextracted osmium fixed human and guinea-pig skin at high magnifications, was reported previously (Matoltsy & Matoltsy 1970; Breathnach, 1971). In contrast, we have since found that guinea-pig plantar epidermal keratohyalin contains larger ribosome-size bodies. It was shown by light microscopy that phospholipid could be removed from keratohyalin granules more easily than from the horny layer by extraction in pyridine, but prolonged fixation in formalin prevented this, probably by strengthening its bonding to protein (Jarrett *et al.*, 1965). For this reason initial glutaraldehyde fixation to stabilize the tissue protein for electron microscopy was kept to a minimum, and might be avoided by use of unfixed frozen methods.

The polar solvents pyridine and ethanol are readily soluble in water, which enables phospholipids, weakly hydrogen-bonded to protein, to be removed by replacement of these bonds by water, after which the lipids are dissolved in the solvent. The non-polar solvents, petroleum ether and chloroform, readily remove free fats (Johnson & Speakman, 1965), but lipids which are bound to protein by hydrogen bonds are less easily removed, unless these linkages are disrupted first by a polar solvent (Gurr & James, 1971). The fact that material was extracted from between the particles in keratohyalin by chloroform shows that here the phospholipid is more weakly associated with protein, possibly by the hydrophobic force which occurs between opposed non-polar groups (Chapman, 1969). Phospholipid is most probably linked by weak hydrogen bonds to protein of the water barrier region in the basal part of the horny layer, since little was removed in non-polar solvents. This is also suggested by the complete removal of phospholipid from the horny cells by polar solvents, which disrupt hydrogen bonds, and by dimethyl sulfoxide, also a weak hydrogen bond breaking agent (Montes *et al.*, 1967). This is the most likely reason why both ethanol and dimethyl sulfoxide are such effective vehicles for penetrating the horny layer barrier.

As keratinized cells are pushed upward into the intermediate and superficial zones of the horny layer, the disjunctum region in light microscopy (Fig. 1), continued enzymic autolysis presumably leads to rupture and breakdown of the lipoprotein complexes, and the contents are normally leached out by transpiration. The absence of bound phospholipid in this outer region was shown by light microscopy (Spearman, 1970), and by a paucity of stainable material, even in the unextracted superficial cells, on electron microscopy (Brody, 1962).

The small particles found in keratohyalin granules after lipid extraction are presumably the protein component demonstrated by Matoltsy & Matoltsy (1970) in isolated granules, and by autoradiography after uptake of labelled amino acids (Fukuyama & Epstein, 1969). Darker peripheral regions to the keratohyalin granules are sometimes observed. This may be due to differences in amino acid composition, since only certain amino acids are strongly stained by osmium (Bahr, 1954). It has been suggested that α-keratin precursor filaments may be derived from ketatohyalin (Odland, 1964), but none was found in keratohyalin in this investigation. Projecting particles can, however, be mistaken for fibres in unextracted tissue viewed under a blanket of phospholipid. The electron dense filaments in the horny cells do not appear to arise from tonofilaments, and they are probably elaborated independently.

The suggestion by Brody (1962) that keratohyalin gives rise to the electron dense material in the basal zone of the horny layer is possibly true, but it has been shown here and in previous work that it is probably phospholipid (Jarrett *et al.*, 1965); it is certainly not matrix keratin, as has been widely assumed (Odland, 1964).

Keratohyalin is clearly heterogenous in composition, with the components only weakly associated, and the various constituents are unlikely to have the same origins and fates. Some components may be cell breakdown products.

The fate of the small particles in the keratohyalin aggregates is the subject of a further paper. They appear to be either dispersed to the horny cell periphery or are autolysed. Keratohyalin is not found in the mouse tail scale epidermis either by light microscopy or electron microscopy, unless it has been treated with retinol (Jarrett & Spearman 1964). Nevertheless, a horny layer is formed, which shows that keratohyalin cannot be an essential factor for keratin formation (Spearman, 1966).

The increased electron density of the plasma membranes when these become thickened is almost certainly due to cystine, which has been demonstrated previously in the horny cell membrane protein by histochemical methods (Jarrett & Spearman, 1964; Spearman & Riley, 1967), by chemical analysis (Matoltsy & Matoltsy, 1966), and by autoradiography (Fukuyama & Epstein, 1969). In the lipid extracted horny cells, matrix keratin may occur as a thin inner lining to the plasma membranes, but it is not found in the cell interiors.

The mechanical strength of the stratum corneum, it is now generally agreed, resides in its tough cystine bonded plasma membranes (Matoltsy & Matoltsy, 1966). The short filaments found in the horny layer appear to be a poorly stabilized form of α-keratin (Baden, 1970) which, devoid of matrix keratin in their interstices, are readily disrupted during paraffin processing for light microscopy, leaving the characteristic large central spaces in the horny cells seen in routine histological preparations. The swollen appearance of the horny cells without discernible intercellular spaces seen by light microscopy resembles the extracted horny layer seen by electron microscopy, and is probably due to diffusion effects when soluble substances are removed by solvents. Brody (1970) showed an irregular distribution of cells in the human horny layer; some with densely packed filaments, and others with empty spaces between dispersed filaments. Probably this is due to individual variation in rates of autolysis within the horny cells, so that some contain more phospholipid than others. Differences in spacing between filaments in different horny cells probably depends on the extent of autolysis of intervening material.

The much greater thickness of the stained peripheries of the horny cells viewed by light microscopy, compared with the ultrastructural appearance, is explained by the fact that sections for light microscopy are some seventy times thicker than those for the electron microscope. Plasma membranes in cells which have been sectioned across when viewed on end will invariably be at a slight tangent in the thicker sections, and in consequence the peripheries will appear to be broader than they really are. Most of the differences shown in the horny layer by the two types of microscopy are therefore resolved.

It is unfortunate that the hair cortical cell, which is markedly different in structure from the epidermal horny layer cell, has been used as a typical model of a keratinized cell by electron microscopists, for in the hair cortex the plasma membranes are not cystine bonded, and mechanical strength resides in the orientated α-keratin filaments embedded in a cystine-rich matrix keratin laid down in the cytoplasm (Gillespie, 1966; Fraser, 1969; Fraser, MacRae & Rogers, 1972). In contrast, cystine does not occur inside the horny layer cells in hairy skin (Crounse, 1963), and the tough cystine bonded plasma membranes are not generally regarded as made up of matrix keratin (Matoltsy & Matoltsy, 1966). Protein reported by several investigators in the interiors of the horny layer cells in hairy skin is presumably derived from the keratin filaments, from the lining cytoplasm, from autolytic products, and possibly from matrix keratin precursor polypeptides broken down before they can be protected by cross linkages. Apart from the dark filaments and lining cytoplasm, they are soluble and are lost from the superficial swollen cells, even in standard methods of electron microscopy. The paucity of keratin in the horny layer cells of hairy skin compared with the hair cortical cells is probably explained by the much greater autolysis of keratin precursor substances, which occurs during keratinization in the former.

Apart from its value in the study of keratinization, the pyridine extraction method, is particularly useful for determining by their electron density whether or not particular cells in the epidermis are keratinized, when examined by electron microscopy.

ACKNOWLEDGMENTS

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Techniques for the replication of skin surfaces

A NEW METHOD

I.E.BARNES

Dental Department, University College Hospital Medical School, University of London

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SUMMARY

Two methods for the replication of skin surface are described. The first is for the macroscopic estimation of hair follicles. The second, which produces replicas for the scanning electron microscope, utilizes Formvar and Thiokol Rubber as the impression material. The replica exhibits fine microscopic detail, and demonstrates sweat gland activity. The technique does not displace the well documented Sylflo method, but may prove to be a useful adjunct.

The use of replication as a technique in the study of surface structure is commonplace in many scientific disciplines. Such techniques may allow the long-term investigation of a specimen, without the necessity of destroying, or removing the specimen from its environment. A series of replicas obtained over a long period may be conveniently examined and compared at the same time.

The present study resulted from a request for the development of a simple method of assessing the density of hair follicles in a standard area of shorn scalp for use in a field study, and describes a new method of replication that has been developed for dental research, and which may prove useful in dermatological studies.

PRINCIPLES OF REPLICATION

An impression is taken of the surface that is to be replicated, and into the negative impression is poured a liquid that is capable of setting to form a hard, durable and accurate replica.

Impression materials

Silicone rubber impression materials are used extensively in dentistry and their use is well known in dermatological studies for the replication of skin surfaces. A monomer, whose viscosity varies according to the proportion of incorporated inert filler, may be polymerized within a few minutes by the addition of a number of drops of a liquid catalyst. Such materials are clean, pleasant smelling and generally inoffensive. When the impression has been taken, the area of the surface of the impression that is to be replicated is demarcated by the construction of a retaining wall, which holds the liquid model material until it has set.

Model material

An excellent and durable model material is embedding Araldite. The formula given below is modified to yield a harder model and a more rapid set than is normally required: Resin CY212, 2 ml; hardener HY 964, 2 ml; accelerator DY 064, 12 drops; plasticiser (di-n-butylphthalate), 3 drops.

The constituents may conveniently be measured with disposable syringes and droppers, into a small stoppered bottle. Following a thorough mixing, the numerous air bubbles are removed by the immersion of the bottle in an ultrasonic cleaner for a period of 8 min. At this stage the liquid Araldite is ready to pour into the impression.

REPLICATION OF SCALP

A silicone rubber, well suited to the replication of the scalp surface for light microscopy, is Cardex-Super, a green material of putty-like consistency. A ball of the putty, about 1·5 cm in diameter, is kneaded with a few drops of catalyst, flattened and supported against a metal disc (or coin), to which it is stuck with the appropriate adhesive. The silicone is pressed firmly against the area of shorn scalp, and then held with minimum pressure until set, at which time it is removed. The retaining wall for the liquid Araldite is applied by sealing a short length of hollow tubing to the impression with wax (Kerr's sticky wax). When the Araldite has set, the Cardex-Super is peeled from the replica, which is presented in an ideal form for storage.

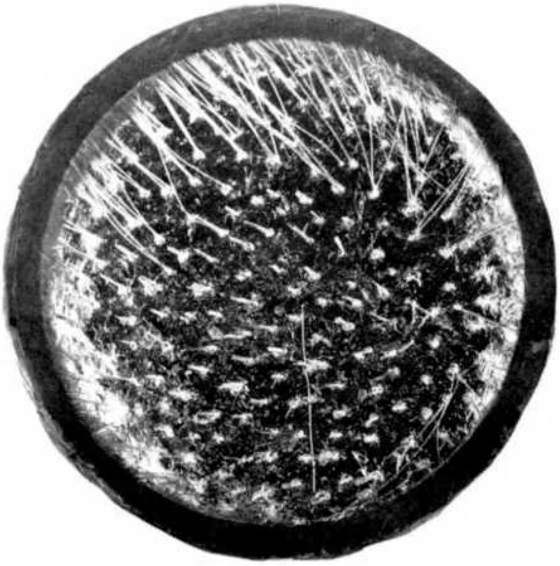

FIGURE 1. Back-lit replica of shorn scalp photographed with a standard ½ plate camera. Cardex-Super impression technique. × 4 life size.

The replica may be examined directly by low power binocular microscope, or photographed. Excellent photographs may be made by utilizing a combination of direct and back lighting which is made possible by the translucency of the model (Fig. 1).

Limitations of the Cardex-Super technique

The viscous Cardex-Super cannot render fine detail at magnifications of greater than × 50, and because of the pressure that is needed to compress and conform the putty-like silicone, only those tissues that are closely supported by bone or cartilage can be replicated without gross distortion.

REPLICATION OF SKIN, SWEAT GLANDS, AND HAIR

The more fluid silicones are capable of yielding replicas of fine microscopic detail, and as they may be flowed onto the skin without pressure they are well suited to lax and unsupported tissues. Impression techniques utilizing Sylflo, a relatively fluid and very useful silicone rubber impression material, have been described in detail by Sarkany (1962), who used nitrocellulose as the model material, and by Johnson, Dawber & Shuster (1970), who used Araldite for the model.

Limitations of the Sylflo technique

The Sylflo impression technique is simple, clean, and quick, but when compared to the solute/solution systems employed in electron microscopy, the material is relatively viscous. This viscosity reduces the capability of reproducing ultra-fine detail.

The Formvar/Thiokol technique

The scanning electron microscope (SEM) is an ideal tool for the study of the surface morphology of skin. It is capable of yielding high definition images with great depth of focus at magnifications of up to × 50,000. With the aid of stereo-pairs, three dimensional images may be obtained, thereby increasing the information that is available. It is these advantages that render the replication techniques used in conventional electron microscopy of such little value for SEM usage. These techniques depend in the main upon the solution of resins or plastics in freely volatile solvents. When such a solution is allowed to flow over a specimen surface, the solvent evaporates leaving a thin pellicle of the solute, which may be stripped from the surface to yield a negative replica. Such replicas are friable, and in the process of stripping they become flattened, losing their surface contour, although retaining their microscopic detail. An example of such a system is 2% Formvar (polyvinyl formal) in chloroform (Formvar 15 95 S, Monsanto Chemicals Ltd).

The technique described below enables a Formvar impression to be removed from a specimen surface in a three-dimensional and morphologically correct configuration, from which a high quality replica may be constructed. Such a replica is well suited to examination in the scanning electron microscope (Barnes, 1972).

Solution. Formvar (polyvinyl formal) 5% in chloroform.

Thiokol rubber. Light-bodied rubber base; light-bodied catalyst; heavy-bodied rubber base; heavy-bodied catalyst; Kerr's Permalastic 150 g tubes (Amalgamated Dental Co.).

The rubber base is supplied in tubes of matched nozzle size, so that strips of equal length of base and catalyst will, when spatulated to a homogeneous mix, set to a firm mass of rubber within 8 min. The heavy-bodied rubber base differs from the light-bodied in that it is more viscous.

Procedure. Two 1 cm strips of light-bodied rubber base and catalyst are mixed on a cardboard slab and transferred to a small deep-sided glass dish. Sufficient drops of the 5% Formvar solution are added to yield, when well spatulated, a freely flowing liquid mix. The number of drops needed to give the required degree of fluidity must be found by experience, but generally falls within the range of 10–20. The resultant mix is poured over the area to be replicated using a clean metal spatula and is left to set (about 10 min). When the impression has set, the pellicle is 'backed' by gently conforming over it a thick layer of the heavy-bodied rubber base. The backing layer bonds with the Formvar/Thiokol impression, and when this layer has set, the composite impression may be removed from the skin surface in a three-dimensional and accurate configuration. Casting Araldite adheres to

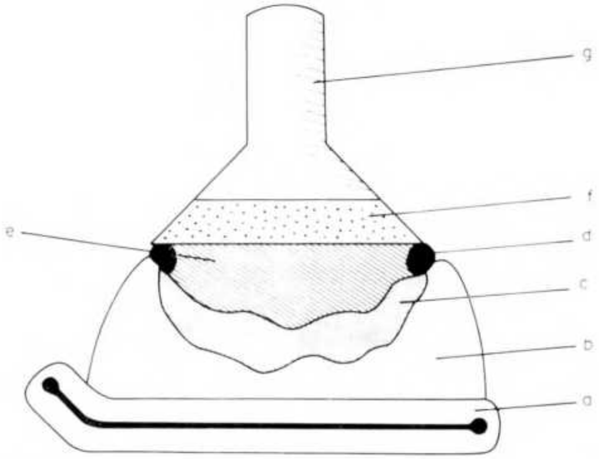

FIGURE 2. Composite cross section of a Formvar/Thiokol impression and Araldite model. The copper ring illustrated as 'section a' is a modification for dental use. Published by permission of the British Dental Journal. Key: b, heavy bodied rubber; c, Formvar/Thiokol; d, retaining wax; e, Araldite; f, adhesive, g, aluminium stub.

conventional rubber base, but not to Formvar/Thiokol. The retaining wall for the liquid Araldite, which is best formed from 'sticky wax' (Kerr Sticky Wax, Amalgamated Dental Co., 26, Broadwick Street, London, W.1.), must be laid down, therefore, within the area of the impression formed by the Formvar/Thiokol if the set replica is subsequently to be easily withdrawn. Before withdrawing the set Araldite, a flat-headed aluminium rivet is glued to the top surface, so that the replica may be mounted in the stage of the scanning electron microscope (Fig. 2).

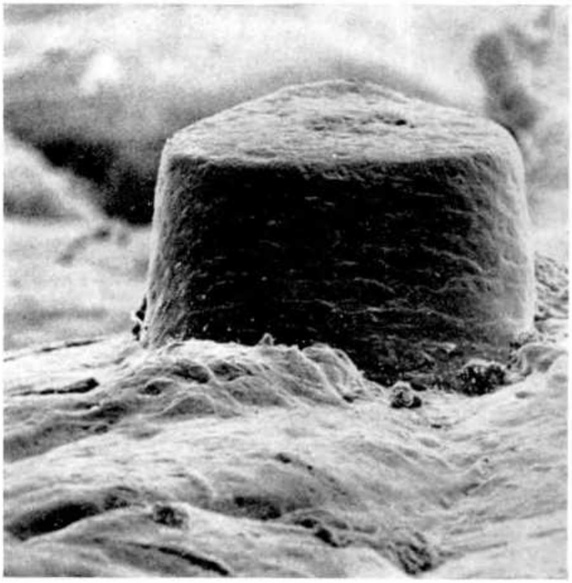

FIGURE 3. Formvar/Thiokol technique. Araldite replica of a shaven hair on the upper lip. Field of view 200 μm.

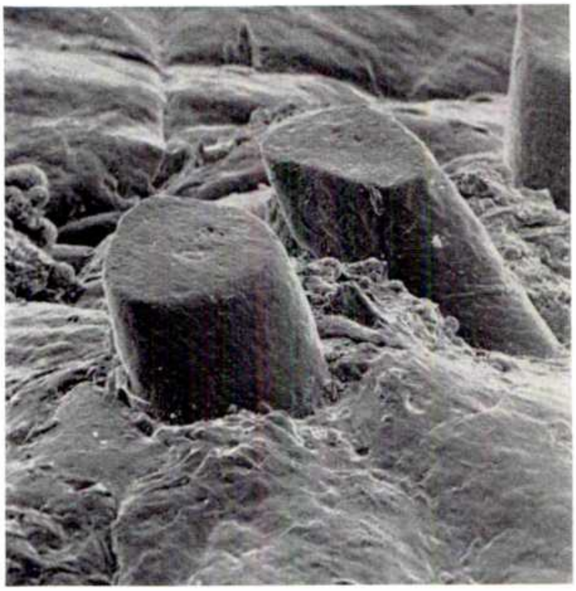

FIGURE 4. Formvar/Thiokol technique. Araldite replica of shaven hairs. Note the torn impression between the middle and posterior hairs. Field of view 400 μm.

Applications of the Formvar/Thiokol technique

Figures 3 and 4 show beard stubble on the upper lip, 8 h after a wet shave. The squames of the skin, the scale structure of the hair and the air spaces within the cortex may be clearly seen.

Figures 5 and 6 show the tip of an index finger. Beads of sweat may be seen exuding from the ducts. This is because the unset impression material is imiscible with, and easily displaced by, sweat. Sarkany has described a method of demonstrating sweat production which involves the transformation of the absorbed chloride in a silicone replica into visible silver (Sarkany & Gaylarde 1968). It might prove possible, by knowing the effective setting time of Formvar/Thiokol and by measuring the volume of the exuded sweat bead, to arrive at a quantitative estimate of sweat production per gland, although many variables would need to be eliminated.

Disadvantages of the Formvar/Thiokol technique

The author has experienced no skin reactions following the application of these impression materials. It would seem sensible to be cautious in their use upon any subject likely to be hypersensitive. The technique is exacting and time consuming.

The set Formvar/Thiokol impression is relatively inelastic and tends to tear when removed from gross undercuts in the specimen surface, into which it flows with ease. A small piece of fractured impression material may be seen between the two hairs shown in Fig. 4 and must be recognized as an artifact.

CONCLUSION

A simple technique for the replication and assessment of hair follicles in the scalp is described. Details are given of a new impression method for the replication of the skin surface in a three-dimensional and accurate form. It is not suggested that this technique (Formvar/Thiokol) will supplant existing methods, but rather that it may prove to be a helpful adjunct.

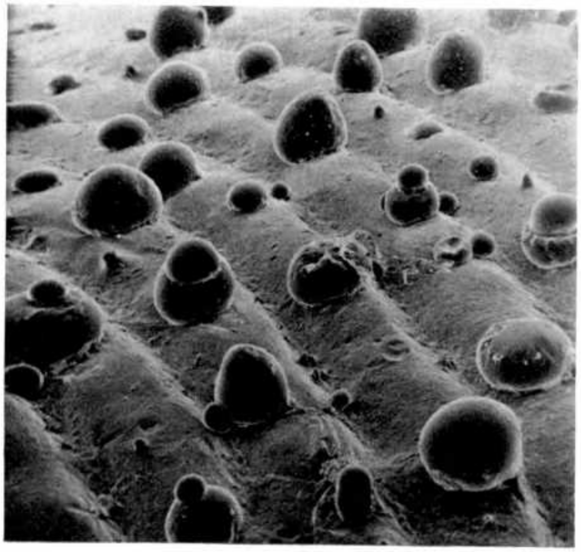

FIGURE 5. Formvar/Thiokol technique. Araldite replica of index finger tip. Note the beads of sweat exuding from the sweat ducts. Field of view 2 mm.

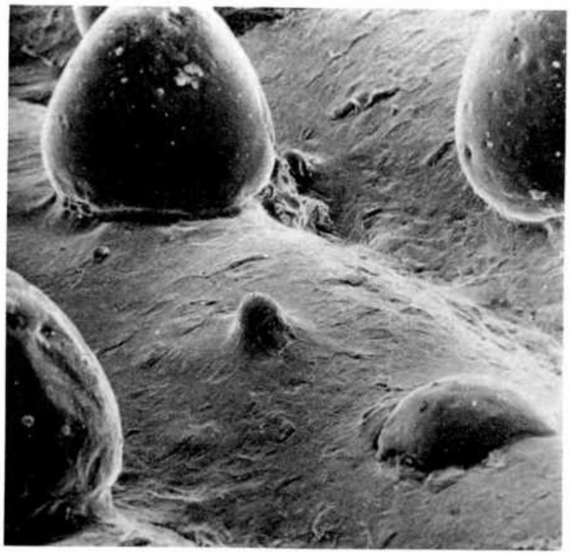

FIGURE 6. Formvar/Thiokol technique. Araldite replica of index finger tip as seen in Fig. 5. Field of view 500 μm

ADDENDUM

The addition of an excess of Formvar solution to the Thiokol (50+ drops) may result in some adhesion of the impression to the model. The optimum number of drops for two 1 cm lengths of rubber base is as stated, 10–20 drops.

ACKNOWLEDGMENTS

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I wish to thank Dr N.J.B.Plomley, Dr A.Jarrett, Dr R.I.C.Spearman and Professor A.S.Prophet for their encouragement; and particularly Dr Alan Boyde, who both advised me and offered me the facilities of his laboratory. My thanks also to Mr P.Reynolds and Mrs E.Bailey for their technical assistance.

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Herpes zoster of the anogenital area affecting urination and defaecation

PETER D. FUGELSO, WILLIAM B. REED, SIDNEY B. NEWMAN* AND JUNE E. BEAMER*

St Joseph's Hospital, Burbank, and* Department of Dermatology, Long Beach VA Hospital, California, U.S.A.

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SUMMARY

Herpes zoster of the anogenital area is reported in six patients. The inability to urinate and to defaecate is a serious complication. This entity is well known in the urological but seemingly unreported in the dermatological literature. None of these patients had a recurrence of the herpes, nor did any have a known neoplasm.

Sacral herpes zoster is apparently seldom seen in dermatological practice, and involvement of the urinary tract with herpes zoster is considered a rare complication. Only twenty-two cases of herpes zoster with bladder symptoms have been reported, all in urological journals (see references).

We are adding six new cases, which seems to stress the desirability of urological evaluation when a patient develops sacral herpes zoster.

CASE REPORTS

Case No. 1

P. A., a 75-year-old white male, was admitted to St Joseph's Hospital with a 5-day history of vesicular skin eruptions associated with sudden onset of nocturia, dysuria and hesitancy. The patient denied having haematuria or foul odour to his urine.

Physical examination revealed grouped vesicular and crusting lesions on the right buttock, thigh and genitalia, corresponding to an S1 to S4 dermatome distribution.

Immediately after admission the patient developed acute urinary retention. After 750 ml of residual urine had been removed, an indwelling Foley catheter was inserted. 10 weeks later the patient was re-admitted for cystoscopy, which showed moderate prostatic obstruction, but no associated bladder lesions. 3 months later a prostatectomy was necessary to relieve the urinary obstruction.

Case No. 2

C. A., a 44-year-old white male, was admitted to the hospital with a grouped vesicular eruption and difficult urination, which had developed 8 days earlier. He denied any haematuria or dysuria.

Correspondence to: Dr Reed, 1013 West Olive Avenue, Burbank, California 91506, U.S.A.

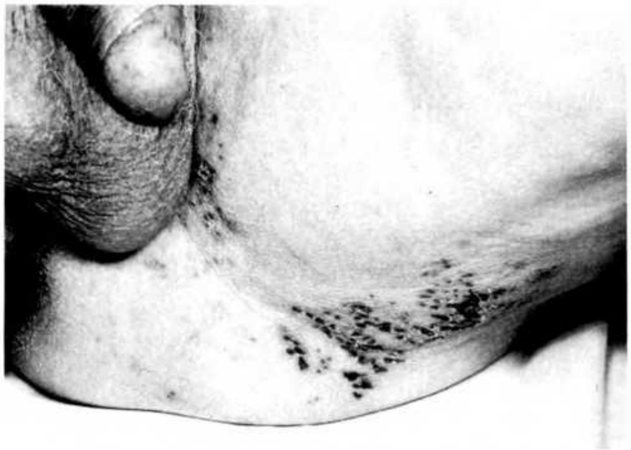

FIGURE 1. Herpes zoster of the lumbar and sacral area in Case 2.

Physical examination showed grouped and scattered vesiculopustular lesions on the right buttock, the back of the right leg, and the genitalia, in an S1 to S4 dermatome distribution (Figs. 1 and 2). Cystoscopy the day after admission showed a normal-appearing prostate, urethra and bladder.

The patient was started on indwelling catheter drainage and oral antibiotics. 1 week after discharge the catheter was removed and the patient was able to void without difficulty. He has remained asymptomatic for the past 2 years.

Case No. 3

E. R., a 73-year-old white male, was hospitalized with a 4-day history of a grouped vesiculo-pustular

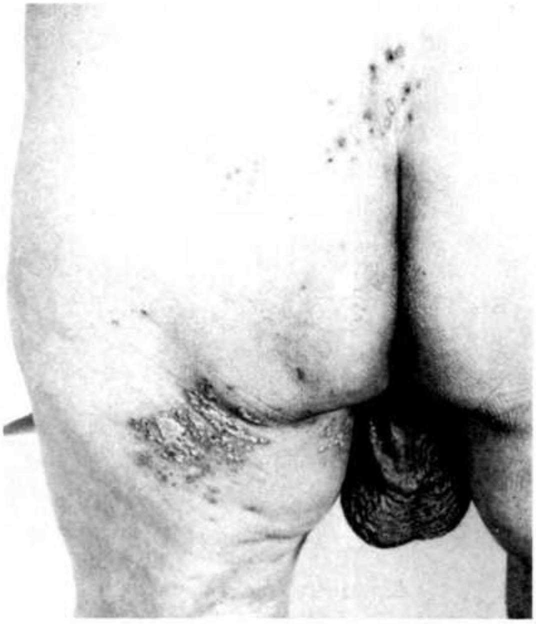

FIGURE 2. Herpes zoster of the lumbar and sacral area in Case 2.

eruption on his right side, involving the buttock, the back of the thigh, and genitalia. He also complained of hesitancy, dysuria, and constipation.

Physical examination revealed grouped vesiculo-pustular lesions in the distribution of the S1 to S4 dermatomes. On rectal examination the sphincter tone was normal and the prostate smooth, non-tender, and only minimally enlarged. Sigmoidoscopy yielded normal findings, except for a small polyp on the right wall 20 cm from the anal opening.

The results of laboratory studies were within normal limits, except for microscopic examination of the urine, which showed 8–10 WBC per high power field and +4 bacteria. Urine contained no protein or sugar and on culture grew β-haemolytic streptococcus, *Escherichia coli* and klebsiella. Serological tests for syphilis were negative. Chest X-ray was within normal limits. Two Giemsa-stained smears from concentrated urine sediment showed many inflammatory cells. No ballooning multinucleated viral giant cells could be seen.

Cystourethroscopy performed 5 days after admission revealed grade 1 obstruction by the lateral lobe of the prostrate, no outlet obstruction, and no vesicular lesions in either urethra or bladder. There were 200 ml of residual urine.

Case No. 4

M. A., a 73-year-old white male, was hospitalized with a 4-day complaint of a vesiculo-pustular skin eruption on the left buttock, thigh and genitalia, corresponding to an S1 to S4 dermatome distribution. On rectal examination the sphincter tone was found to be normal and the prostate small, smooth, rubbery, and non-tender.

Histological examination of a 3 mm punch biopsy specimen of involved skin from the left thigh showed multilocular vesicle formation and degenerating epithelial cells, with amorphous eosinophilic material replacing the nuclei. These eosinophilic intranuclear particles represented viral inclusion bodies (S-2726-65).

Laboratory evaluation demonstrated a WBC of 7,500, with 86% neutrophils, 12% lymphocytes, and 2% eosinophils. The ESR was 30 mm in 1 h. Microscopic examination of the urine showed 2–3 epithelial cells and 4–5 red cells per high power field. There was no protein or sugar in the urine. The serological tests for syphilis were negative. The residual urine was 400, 350, and 90 ml on three occasions.

The patient was placed on indwelling catheter drainage and given 1 g of methenamine mandelate four times daily. Cystourethroscopic examination after 8 weeks of catheter drainage showed no vesicles or obstructive prostatic tissue. However, patchy erythema of the bladder fundus, secondary to catheterization, was visible. The catheter was removed a week later and the patient voided without difficulty. 1 day later his residual urine was 25 ml after voiding 400 ml.

Case No. 5

G. B., a 76-year-old white female, sought help from a urologist for severe dysuria. 2 days after the onset of the dysuria she had developed herpes zoster of the vulva and left buttock, but without any history of pre-zoster type pain. She suffered urinary and faecal retention for 3 weeks. This episode resolved without specific treatment, but she has had severe post-herpetic neuralgia for the subsequent 6 months.

Case No. 6

M. G., a 75-year-old white male, had a transurethral resection of the prostate in 1957, and another in 1967. He had virtually no urological symptoms until 3 days before the onset of sacral herpes zoster, when he developed urinary retention and difficulty in defaecation, but no pre-zoster type pain.

These problems resolved in a week, and the herpetic lesions cleared in 3 weeks without post-herpetic neuralgia.

DISCUSSION

In herpes zoster of the anogenital region, both motor and sensory nerves may be involved. The sphincters of the urethra and anus are controlled by motor nerves from the sacral plexus. The exact pathophysiology leading to the urinary and faecal retention in the cases described is not clear, but it is obvious that the end organs themselves need not be involved with vesicular lesions. The situation is similar in herpes zoster affecting the cranial nerves, when the motor nerves may also be affected.

Clinically the patients had herpes zoster, but virological studies to ascertain whether the vesicular lesions were due to varicella or herpes simplex type II were not available. However, none of the patients has had recurrent lesions typical of herpes simplex.

One of us (W.B.R.) has noted painful zosteriform herpes of lumbar distribution following low back surgery in three patients. In two of the six patients reported, the urological complaints preceded the pain and lesions of the herpes zoster, and it is interesting to speculate that the zoster may have been precipitated by their pre-existing urological problems.

The authors hope that this report will alert dermatologists to anticipate, in patients with sacral herpes zoster, the appearance of such urinary symptoms as acute retention, and of bowel symptoms such as faecal retention.

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Eczéma craquelé as the presenting feature of myxoedema

ANDREW P. WARIN*

Department of Dermatology, St Thomas' Hospital, London, S.E.1

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SUMMARY

Three cases of advanced myxoedema are reported who presented with Eczéma craquelé.

Brocq (1907) used the term Eczéma craquelé to describe the picture produced when the stratum corneum dries out and cracks giving a crazy-pavement pattern to the skin, which becomes itchy and eczematous. He also used an alternative name of Eczéma fendillé.

Kreibich (1927) used the same term and pointed out that when the stratum corneum dries out and fissures, the patient rubs the skin and thus irritates it more and so sets up a vicious circle. He noticed that lichenification often resulted and pointed to chemical irritation, woollen clothing, blankets and horse hair as initiating factors.

Gross (1941) used the term Eczéma rhagadiforme, and pointed out that nummular or discoid eczema often becomes superimposed on it. He noted that cold weather, the use of alkaline soaps and chemicals, and excessive bathing all played a part in the development of this condition.

Blank (1952) pointed out that the water content of the horny layer of the skin is determined by the relative humidity of the environment, and that the skin in Eczéma craquelé is similar to that seen in chapped hands when there is excessive water loss from the stratum corneum.

Degos (1962) also referred to this condition as Eczéma craquelé and described how the cracking occurs in an uneven diamond pattern with mild scaling, and with subsequent eczema spreading out from the fissures. He noted that it responded to wool fat (Lanolin).

Caplan (1970) described nine patients under the title—superficial haemorrhagic fissures in the skin. He also mentioned the terms erythème craquelé and eczema craquelatum and état craqueelé. One of his patients had myxoedema and six of them were receiving diuretic drugs.

Domonkos (1971) uses other names for this condition: winter itch, winter dry skin, asteototic eczema or xerotic eczema. He describes it mainly on the shins of the elderly, and as looking like cracked porcelain. Rook & Wilkinson (1972) prefer the term asteatotic eczema and mention xerotic eczema and eczema hiemalis as alternatives. They also point out that nummular eczema may commonly supervene.

CASE REPORTS

Case 1

A man aged 70 presented with a 1 year history of generalized itching and dryness of the skin. He had been treated with various topical corticosteroid preparations, with some temporary benefit.

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* At present Senior Registrar, Skin Department, The London Hospital.

On examination his skin generally was very dry, but on the arms and legs showed well marked crazy-pavement fissuring and a superimposed discoid eczema. It was noticed that his voice was deep and husky and his pulse slow, and that the relaxation of his ankle jerks was very slow. His face showed a yellowish oedema and he complained of always feeling cold.

Investigations. Serum cholesterol, 365 mg/100 ml; PBI, less than 1 μg/100 ml; plasma thyroxine level, zero; ECG, low-voltage and flat T-waves.

Case 2

A man aged 47 presented with a year's history of dry and itchy skin on his arms and legs. He had used betamethasone valerate ointment, with some relief of symptoms, but without lasting benefit.

On examination he was in an advanced state of myxoedema. His skin was very dry all over, and on his arms and legs he had crazy-pavement fissuring with superimposed discoid eczema and some lichenification.

Investigations. PBI, 1·1 μg/100 ml; ECG, low voltage and flat T-waves.

Case 3

A female aged 80 was referred because of a generalized itchy rash of 6 months duration, which had not responded to topical corticosteroid preparations.

On examination she was myxoedematous. Her arms and legs showed crazy-pavement fissuring and discoid eczema. She had numerous scratch marks and some lichenification.

Investigations. Total thyroxine, 2·3 μg/100 ml (normal 5·3–12·5 μg/100 ml).

All these patients were treated with 1-thyroxine and within 3–4 weeks their skin appeared normal. They were all at first given beclomethasone dipropionate 25% in white soft paraffin and aqueous cream as a soap substitute, but once their myxoedema was reversed topical treatment was discontinued yet they remained free from Eczéma craquelé. None of these patients had a previous history of eczema or atopy.

DISCUSSION

The first change in the development of Eczéma craquelé is dryness of the skin, and this may be entirely symptomless. However, the keratin layer tends to crack and crazy-pavement fissuring occurs, and the fissures between the pavings tend to become irritated and reddened. It is often then intensely itchy. It is then common for discoid eczema to spread out from the irritated fissures.

The following factors may play a part in the development of Eczéma craquelé: (1) a naturally dry skin; (2) decreased sweat and sebaceous gland activity in the elderly; (3) decreased keratin synthesis in the elderly; (4) degreasing by solvents and cleaners, both industrial and domestic; (5) low environmental humidity and dry, cold winds; (6) loss of integrity of the water reservoir of horny layer; (7) protein depletion and wasting diseases; (8) strong diuretics; (9) friction—it is commonly seen over the knees of elderly patients in hospital.

It may well be that a number of these factors must operate together to produce Eczéma craquelé. For example, in the elderly, it may be the dry, cold wind and low humidity that precipitate the gross changes, but predisposing factors are already present. In low humidity with a dry, cold wind, the stratum corneum dries out and fissures and may develop Eczéma craquelé. In myxoedema, there is diminished sweat gland activity and probably also diminished sebaceous gland activity. However, the epidermal water-barrier and transepidermal water loss are normal. It may well be that the water-holding capacity is low (Grice, 1972).

A dry skin and crazy-pavement fissuring, with or without eczema, are both commonly seen in myxoedema, but no report has been traced of Eczéma craquelé as the presenting feature of this disease. Myxoedema is often a difficult diagnosis to make, mainly because its development is insidious. Eczéma craquelé is a common condition and it is only too easy to overlook the possible role of general medical factors in its causation. The purpose of this paper is to draw attention to the fact that it may be a presenting feature of myxoedema.

ACKNOWLEDGMENTS

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Pustulosis palmaris et plantaris

KRISTIAN THOMSEN AND POUL ØSTERBYE

Department of Dermatology, Finsen Institute, Copenhagen, Denmark

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SUMMARY

Forty patients with pustulosis palmaris et plantaris were treated with tetracycline 250 mg twice daily or with placebo, in a double-blind trial. In twenty-eight patients the lesions were confined to the palms and soles, whereas twelve had psoriatic lesions affecting other parts of the skin and/or nails. Radiological signs of focal infection were found in eighteen patients. In thirty-five patients positive bacteriological culture from pustules was obtained, the most common strain being *Staphylococcus albus.*

Tetracycline proved superior to the placebo ($P<0.001$) in improving the lesions of PPP. It is discussed whether the effect of tetracycline is due to an antibacterial mechanism.

Pustulosis palmaris et plantaris (PPP) is a familiar disease, well known to be refractory to treatment. Various treatments have been tried, including methotrexate, which appeared to have some effect, especially in cases having also psoriasis of the nails or elsewhere on the skin (Thomsen, 1971).

Tetracycline has a partly unexplained effect upon many pustular conditions. However, to the best of our knowledge no controlled study of the effect of tetracycline in PPP has been published. We are, therefore, reporting the results of oral tetracycline treatment of PPP in a double-blind study.

MATERIAL

Forty consecutive patients with PPP were the subjects of this study. The disease affected the palms and soles and was characterized by crops of pustules with scaling and redness, accompanied by itching and tenderness. Twelve patients had psoriatic lesions in other areas of the skin or nail psoriasis. In the remaining twenty-eight patients only the palms and soles were affected.

Sex

Thirty-two females and eight males.

Age

At the time of the study the patients ranged in age from 16 to 78 years, mean 45·7 years.

Duration of disease

When the treatment was started the disease had lasted 3 months to 50 years, mean duration 4·7 years.

In eighteen patients the disease had lasted for less than 1 year, and in five patients the duration of disease exceeded 10 years.

Method

Before instituting treatment the following tests were performed: Hb, ESR, WBC, differential count, antistreptolysin O titre, urine analysis, radiography of the jaws and sinuses. Furthermore, patch testing was carried out according to the routine of the Finsen Institute; bacteriological study of the pustules, and direct mycological examination and culture of scrapings from pustules. Photographs were taken of the palms and soles.

Treatment

A double-blind technique was applied. Tetracycline, 250 mg, and identical-looking lactose capsules were used, the dosage being one capsule twice daily.

The treatment was randomized in three periods of 4 weeks each. Thus, each patient had one course of tetracycline and two courses of placebo or *vice versa*. The treatment periods followed immediately upon each other, but the sequence was accidental.

The only topical treatment was a hydrated petrolatum (vaselinum aquosum of the Danish Pharmacopoeia). All patients were examined by one of the authors at the end of each treatment period. The extent of the lesions was measured, the number of pustules counted, and photographs were taken.

The therapeutic results were evaluated as + response or 0 response, + response indicating that the lesions had disappeared completely or had considerably diminished, with pustules no longer present.

RESULTS

Of the forty patients treated thirty-eight completed the study. Two patients stopped taking the drug after two courses because of side effects. Nevertheless, these two patients are included in the analysis.

Table 1 gives the results of treatment with tetracycline and placebo. Tetracycline was administered in fifty-nine courses and placebo in fifty-nine. Tetracycline proved effective in a total of forty-two courses, i.e. diminution or disappearance of the lesions, while placebo had a similar positive effect in twenty courses. Tetracycline was ineffective in seventeen and placebo in thirty-nine courses. This difference between the effect of tetracycline and placebo upon forty patients with PPP is highly significant ($P < 0.001$).

TABLE 1. Results of treatment with tetracycline 500 mg daily compared with placebo. The study includes forty patients. Two patients stopped treatment after two periods

		Period			
		1	2	3	Total
Tetracycline	+ response	14	13	15	42
	0 response	6	6	5	17
Placebo	+ response	7	6	7	20
	0 response	13	15	11	39

There was no difference between the effects of tetracycline and placebo according to whether the drugs were given in the first, second, or third course.

The twelve patients having psoriatic lesions elsewhere did not differ in their response from the remaining patients whose lesions were confined to the palms and soles.

Furthermore, no relationship was found between the rate of response, age, sex, or duration of the disease.

Radiographic signs of focal infection were found in eighteen patients (teeth, twelve patients; sinuses, six patients), but these patients responded in the same way as the others.

In thirty-five patients a positive bacteriological culture from the pustules was obtained. In nine patients *Staphylococcus aureus* was found, and in one patient haemolytic streptococci, but in twenty-six cases only *S. albus* was isolated. The anti-streptolysin O titres were normal and the Hb, ESR, WBC, and differential counts within normal limits in all the patients.

Patch tests were positive in six patients: cobalt in two patients, nickel in one, neomycin in one, formalin in one, and benzyl salicylate in one patient.

Side effects, viz. nausea and/or diarrhoea, were reported by eight patients in the tetracycline group and by three in the placebo group.

DISCUSSION

From this study it is evident that tetracycline has an effect upon PPP independent of the presence or absence of focal infection or of psoriatic lesions elsewhere on the skin.

We are not claiming that tetracycline will cure a case of PPP, but are reporting only that tetracycline, 250 mg twice daily, is significantly superior to a placebo in the treatment of PPP. In some cases the lesions disappeared completely, but in most cases only an improvement was recorded. However, according to this study there is no doubt that tetracycline orally is a valuable adjuvant in the treatment of this highly refractory disease.

The mechanism of this tetracycline effect is unknown. It might be some specific dermatological, non-antibiotic effect, but the finding of bacteria in the pustules in thirty-five out of forty cases might suggest a true antibacterial effect, although *S. albus*, found in twenty-six cases, is normally considered a non-pathogen. The pustules of pustular psoriasis of von Zumbusch are also claimed to be sterile, but nevertheless systemic antibiotic therapy appears to have an effect upon this disease (McFadyen & Lyell, 1971). However, the question remains whether the bacteria found in PPP are in fact pathogenic or only represent the normal skin population.

Radiological signs of focal infection in eighteen out of forty patients is more than we expected to find in a normal population, but on a level with that found by others in PPP (Enfors & Molin, 1971; Hellgren & Mobacken, 1971). This revives Andrews' concept of pustular bacterid (Andrews & Machacek, 1933). However, no difference in response to tetracycline was found whether or not radiological signs of focal infection were present. Admitting that radiological examination does not disclose all septic foci, the effect of tetracycline can, therefore, hardly be ascribed to an effect upon a bacterial focus, but rather to an unknown tetracycline action in the skin.

Tetracycline has been used in acne vulgaris for almost 20 years, and the mechanism of its action is believed to be an antibacterial effect upon *Corynebacterium acnes*, with reduced hydrolysis of triglycerides. Tetracycline is even more effective in rosacea (Sneddon, 1966; Wereide, 1969), although this condition is not believed to be caused by micro-organisms. However, it has recently been demonstrated that ampicillin is nearly as effective as tetracycline in the treatment of rosacea, which perhaps indicates that bacteria do play some role in this disease (Marks & Ellis, 1971).

By analogy, the effect of tetracycline on PPP might be antimicrobial. Studies using other antibiotics would be able to confirm this hypothesis.

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Pharmacology and Treatment

Comparison of clotrimazole cream, Whitfield's ointment and Nystatin ointment for the topical treatment of ringworm infections, pityriasis versicolor, erythrasma and candidiasis

YVONNE M. CLAYTON AND BRUCE L. CONNOR*

St John's Hospital for Diseases of the Skin, London WC2H 7BJ

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SUMMARY

A double-blind trial was used to compare the efficacy and acceptability of topical treatment with clotrimazole cream or Whitfield's ointment for ringworm, pityriasis versicolor, and erythrasma infections, and that of clotrimazole cream or Nystatin ointment for Candida infections. Clotrimazole was found to be as effective and acceptable as Whitfield's ointment and Nystatin ointment.

The comparison of the efficacy and acceptability of topically applied chemical agents and antibiotics in skin diseases is one of the most difficult exercises to submit to statistical analysis. There are many factors which make such comparisons a problem, among these being the selection of similar cases, the availability of such cases in sufficient numbers, the occurrence of spontaneous remissions and, in the case of fungal infections, variations in degree of colonization of the skin. Nevertheless, unless a drug has a most dramatic effect when applied to the skin surface, there is no alternative but to plan careful double-blind trials.

Clotrimazole (Fig. 1), a tritylimidazole derivative, is a new drug with a wide spectrum of antifungal activity. *In vitro* fungistatic activity is shown against the dermatophytes (ringworm fungi), Candida and other yeast species, Aspergilli, and many of the organisms responsible for subcutaneous and systemic mycotic infections. It is also active against some Gram-positive bacteria. Clotrimazole can be given orally and has been used in the treatment of generalized candidiasis (Marget & Adam, 1971), pulmonary and disseminated cryptococcosis, and aspergillosis. However, the potential of a topical preparation effective not only against dermatophytes but also against yeasts, seemed to warrant a comparative trial against standard treatment, such as Whitfield's and Nystatin ointments respectively.

METHODS

During a 4-month period, all patients seen in the mycology department found to be positive on microscopy of skin scrapings for either ringworm fungi, *Malassezia furfur*, Corynebacteria, or Candida

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* Present address: St Thomas' Hospital, London SE1 7EH.

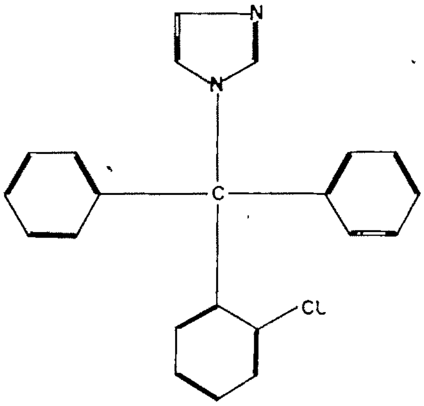

FIGURE 1. Structural formula of clotrimazole (1-α-2-chlorophenylbenzhydryl imidazole).

yeasts, were referred to one clinician (B.L.C.) for his assessment of suitability for inclusion into the trial. The patients were always seen by the same clinician on their return visits to the hospital. Patients whose first skin scrapings were subsequently found to be negative on culture were excluded from the trial.

The patients were randomized to treatments A or B for dermatophyte, pityriasis versicolor, or erythrasma infections, and to C or D for Candida infections. Neither the clinician, the mycologist, the pharmacist, nor the patient, knew which preparation was being used. The clotrimazole cream (A and C) was supplied by Bayer Pharmaceuticals Ltd, each 20 g tube of 1% cream containing:

Clotrimazole pure substance	0·2 g	Lanette O	2·0 g
Arlacel 60	0·4 g	Eutanol G	2·7 g
Tween 60	0·3 g	Benzyl alcohol	0·2 g
Spermaceti	0·6 g	Distilled water to	20·0 g

Whitfield's ointment (B) as supplied by Evans Medical, Liverpool, contained 3% salicylic acid and 6% benzoic acid in an emulsifying ointment. Nystatin ointment (D) as supplied by E.R.Squibb and Sons Ltd, Twickenham, contained 100,000 units of Nystatin per g in Plastibase. All patients were instructed to apply the ointment twice daily to the affected areas; treatment was to be continued for 4 weeks. Clinical assessment and mycological examinations (both by direct examination of skin scrapings and by culture) were carried out on all sites to which treatment had been applied after 2 and 4 weeks of treatment. Patients were asked to stop treatment for 24 h before coming for their 2- and 4-week assessment tests. A clinical and mycological reassessment was made at 8 weeks, patients having had no therapy for 4 weeks.

The aim of the trial was to treat thirty patients in each of the four disease groups, so that a statistical analysis could be applied to the results. This was achieved for cases of ringworm and pityriasis versicolor infections: but although patients with erythrasma and Candida infections continued to be accepted for a further 2 months, after the initial 4 months, only twelve suitable cases of erythrasma were seen, of whom eight completed the trial, and only twenty-three cases of Candida infections were accepted, of whom seventeen completed the trial.

The number of infected sites on each patient was recorded and the results on follow-up were scored both for microscopy and culture as: 1, organism present; ½, organism scanty; 0, organism absent.

In all groups, the number of sites treated was comparable.

The clinician's opinion as to effectiveness was scored as: 1, good; ½, partial; 0, none.

The patient's opinion on acceptability was scored as: 1, good; ½, moderate; 0, poor.

RESULTS

Ringworm infections

A total of forty-three patients with ringworm infections of the skin were admitted into the trial; of these thirty-two completed 8 weeks in the trial. Sixteen of these patients were using clotrimazole and sixteen Whitfield's ointment.

Sex and age. There were three females compared to twenty-nine males. Ages ranged from 13 years to 63 years, approximately 60% of the patients being between 20- and 40-years-old. The two groups being compared had similar values for these two characteristics:

	On clotrimazole	On Whitfield's
Sites involved		
Feet	4	6*
Feet and body	0	3
Feet and groins	7	5
Groins	4†	2
Groins and body	1	0
Fungus isolated		
Trichophyton rubrum	14	13
T. mentagrophytes var. *interdigitale*	2	2
Epidermophyton floccosum	0	1

* One also with leg involvement; † two also with buttock lesions.

Results. The percentage of patients for which infection was detected by microscopy and by culture in the two treatment groups after 2 and 4 weeks therapy, and after a further 4 weeks without treatment, are shown in Table 1. No significant difference between clotrimazole and Whitfield's ointment was found after making tests to compare pairs of percentages. Of the six patients in each group who were culture positive at the end of the trial, four on clotrimazole and three on Whitfield's were mycologically negative after the 4 weeks of therapy, but had relapsed during the 4-week period without treatment. The other five culture positive patients at the end of the trial had all given positive mycological findings throughout the treatment period.

Pityriasis versicolor

Of the thirty-five patients who started treatment in the trial, thirty-one completed the course of

TABLE 1. Ringworm infection: percentage of patients for which infection was detected by microscopy and by culture at any site

	Clotrimazole (Sixteen patients)		Whitfield's (Sixteen patients)	
	Microscopy	Culture	Microscopy	Culture
At 2 weeks	56·2	43·7	81·2	31·2
At 4 weeks	37·5	12·5	43·7	12·5
At 8 weeks	56·2	37·5	50·0	37·5

therapy and returned for reassessment after 4 weeks without treatment. Seventeen patients were treated with clotrimazole and fourteen with Whitfield's ointment.

Sex and age. The ratio of males to females was 2:1. Two-thirds of the patients were between 20- and 30-years-old.

Sites involved. Nearly all the lesions were on the trunk, sometimes extending to the neck and arms. One patient also had a lesion on a knee. *Malassezia furfur* was demonstrated in all skin scrapings.

Results. The percentage of patients for which infection was detected by microscopy at any site is shown in Table 2. Nearly half the patients in each group had cleared after 2 weeks treatment as assessed by microscopical examination of skin scrapings. After 4 weeks using clotrimazole, all but two patients were free from infection, both clinically and microscopically; four patients on Whitfield's ointment remained infected after 4 weeks. No statistically significant differences in response to therapy were found.

TABLE 2. Pityriasis versicolor: percentage of patients for which infection was detected by microscopy at any site

	Clotrimazole (Seventeen patients)	Whitfield's (Fourteen patients)
At 2 weeks	52·9	50·0
At 4 weeks	11·8	21·4
At 8 weeks	11·8	14·2

TABLE 3. Erythrasma: percentage of patients for which infection was detected by microscopy and by culture at any site

	Clotrimazole (Four patients)		Whitfield's (Four patients)	
	Microscopy	Culture	Microscopy	Culture
At 2 weeks	75	50	50	0
At 4 weeks	0	0	0	0
At 8 weeks	25	25	50	25

Erythrasma

Only eight patients were included in the trial, four being treated with clotrimazole and four with Whitfield's ointment. All the patients were males aged between 33 and 65, all had lesions in the groins, and two patients also presented with axillary infection. *Corynebacterium minutissimum* was isolated from all cases.

Results. These are shown in Table 3. All patients were clinically and bacteriologically free from infection after 4 weeks treatment. No differences were detected between those using clotrimazole and those using Whitfield's ointment.

TABLE 4. Comparison of effectiveness and acceptability of clotrimazole and Whitfield's ointment on cases of ringworm infections, pityriasis versicolor, and erythrasma, after 4 weeks treatment

	Mean percentage	
	Effectiveness	Acceptability
Clotrimazole	0·98	0·97
Whitfield's	0·93	0·89

Scored as: effectiveness; good, 1; partial, ½; nil, 0: acceptability; good, 1; moderate, ½; poor, 0.

Comparison of effectiveness and acceptability of clotrimazole and Whitfield's ointment

There were no significant differences between the efficacy of clotrimazole and Whitfield's ointment, as assessed by the clinician. Similarly, acceptability and the presence of side effects, as assessed by the patient, revealed no significant differences between clotrimazole and Whitfield's ointment. The results, combining the findings of the three disease groups are shown in Table 4.

Candidiasis

Seventeen patients completed 8 weeks in the trial, eight of whom were treated by clotrimazole and nine by Nystatin.

Sex and age. There were two females compared to fifteen males; aged between 17 and 50 years.

Sites involved. The groin was affected in all but three cases, with perianal involvement in five of them. One patient had lesions in the groin and axillae, and another in the groins and toe spaces. Two patients had perianal lesions only, and one solely in the axilla.

Candida albicans was the organism responsible for all the skin infections.

TABLE 5. Candida infections: percentage of patients for which infection was detected by microscopy and by culture at any site

	Clotrimazole (Eight patients)		Nystatin (Nine patients)	
	Microscopy	Culture	Microscopy	Culture
At 2 weeks	12·5	12·5	33·1	11·1
At 4 weeks	0	0	0	0
At 8 weeks	0	0	0	0

Results. All seventeen patients were clinically and mycologically free from infection after 4 weeks therapy; no relapses occurred after 4 weeks without treatment. No significant differences were found between clotrimazole and Nystatin (Table 5). Their effectiveness and acceptability as assessed by clinician and patient respectively were similar (Table 6).

DISCUSSION

The results of this double-blind trial show that clotrimazole is as effective as Whitfield's ointment for

TABLE 6. Candida infections: comparison of effectiveness and acceptability of clotrimazole and Nystatin, after 4 weeks treatment

	Mean percentage	
	Effectiveness	Acceptability
Clotrimazole	0·94	1·00
Nystatin	0·94	0·89

Scored as in Table 4.

the treatment of ringworm infections of the glabrous skin, pityriasis versicolor, and erythrasma infections. It is also as effective as Nystatin for the treatment of Candida skin infections. This broad spectrum of activity may well prove beneficial when facilities for accurate mycological diagnosis are not readily available.

The selection of patients for the trial was largely dependant on their knowledge that they would be able to attend for assessment at the required times and after 8 weeks from the start of treatment. This naturally ruled out some suitable patients, and made it difficult to obtain adequate numbers for analysis in the case of erythrasma and Candida infections. The co-operation of the patients was, nevertheless, rather greater than we had anticipated, particularly for those who returned 4 weeks after having had no treatment. The majority of the patients said that they had had their infections from between 6 months and 2 years, although the duration ranged from a few weeks to 20 years for one patient. Nearly half of the patients with ringworm infections had recently been using fluorinated corticosteroid preparations (Betnovate or Synalar).

The predominance of *Trichophyton rubrum* as the causative organism of the ringworm infections in this series of patients may partially explain the rather slow response of the skin lesions to topical therapy with either clotrimazole or Whitfield's ointment. However, after 4 weeks treatment all but two patients in each group were negative as assessed by culture for ringworm fungi. All the strains of ringworm fungi isolated from the patients before treatment was started were sensitive to clotrimazole, having minimum inhibitory concentrations between 0·35 and 1·5 μg/ml. None of the strains isolated after treatment was stopped were shown to have developed resistance to clotrimazole. No differences were detected in the efficacy of treatment by either clotrimazole or Whitfield's ointment on various sites; feet and groins responding equally well to both preparations.

As in the trial using topical treatment with clotrimazole reported by Fredriksson (1972) there were no reports of allergic contact dermatitis nor of photosensitization. The only side effects encountered were transient burning and irritation, occurring mostly immediately after application following bathing. This effect was reported by seven of the forty-five patients on clotrimazole, and by ten of the thirty-four patients using Whitfield's ointment.

ACKNOWLEDGMENTS

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Case Reports

Acute lymphangitic nocardiasis

E. GEZUELE, I. A. CONTI-DÍAZ, F. BURGOA AND ANA C. DE JIMÉNEZ

Department of Parasitology (Mycology Section), Institute of Hygiene,
School of Medicine, Montevideo, Uruguay

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SUMMARY

The third case of lymphangitic nocardiasis caused by *Nocardia brasiliensis* to be recorded in Uruguay is presented.

The clinical picture showed some of the features of sporotrichosis, but it was more acute, the nodules developing rapidly into abscesses.

A review of eight similar cases, reported previously, reveals that *N. brasiliensis* was properly identified in five of them. All cases were localized on the upper limbs, and granules were not seen on direct examination.

Mycetomas caused by *N. brasiliensis* have not been observed in Uruguay.

Reports of nodular nocardiasis of the upper limbs are rare (Guy, 1920; Alarcón, Obadia & Borelli, 1958; Raich, Casey & Hall, 1960; Baumgarten, 1961; Rapaport, 1966; Moore & Conrad, 1967). The first two cases observed in Uruguay from which *Nocardia brasiliensis* was isolated, were published by Mackinnon *et al.* (1968). This paper describes a third.

CASE REPORT

A white man aged 29 was seen in consultation 2 weeks after noticing a pustular lesion on the left forearm. He was employed in a rural area trimming trees and digging, but could not recall any recent wound or trauma. After squeezing the lesion, a slightly painful cellulitic area appeared around it, accompanied by axillary tenderness. The patient was treated with tablets of oxytetracycline for 10 days without improvement. On the contrary, the lesion became ulcerated and several painful nodules developed along the ventral side of the arm.

The examination revealed an ulcer 2 cm in diameter, surrounded by a 6 cm erythematous plaque on the upper anterior aspect of the left forearm; just above the ulcer there was an indurated erythematous nodule 3 cm in diameter. Three smaller nodules on the ventral side of the arm were connected by a lymphangitic cord, 5 mm thick, which extended to the axilla. The nodules were exquisitely tender with ill-defined borders. Central fluctuation was evident.

The blood picture, sedimentation rate, urinalysis, and chest X-ray were normal.

Mycology

The pus obtained by puncture of one of the nodules did not show granules. Smears stained by

Gram's method revealed scanty, branched, Gram positive hyphae, 1 μm in diameter. Cultures on brain–heart infusion agar slants developed multiple colonies that were identified as *N. brasiliensis*, following methods described in another paper (Conti-Díaz *et al.*, 1971). Its thermotolerance was established between 41 and 43°C. The strain was inoculated intratesticularly in a guinea-pig and intraperitoneally in five male white Swiss mice. The guinea-pig was killed 34 days after inoculation and showed testicular abscesses with actinomycetic hyphae. The mice killed at the same time had nodules on the omentum containing granules 250–300 μm in diameter without clubs. *N. brasiliensis* was re-isolated from all the animals.

Treatment

The patient was treated with tablets of sulphadiazine, 1·5 g every 6 h. A week later there was a notable regression of the lesions. The ulcer healed in 10 days, but the lymph nodes of the arm were still evident 3 weeks after starting the treatment. 2 weeks later all the lesions had healed. The patient did not return for follow-up.

DISCUSSION

Mycetomas caused by *N. brasiliensis* have not been observed in Uruguay. Nevertheless, three cases of acute lymphangitic nocardiasis from that species were seen in a relatively short period of time. We think that lymphangitic nocardiasis might represent an early stage of infection that, due to prompt diagnosis and treatment, does not evolve to the stage of mycetoma, which is usually recognized several months after the onset of symptoms. According to Latapí (1959) and Latapí & Ortiz (1963), among 197 cases of mycetomas caused by different aetiological agents, only sixteen sought medical assistance within a year after the beginning of symptoms. Some of them probably were of longer duration, since they already presented well-developed bone lesions.

In all cases of acute lymphangitic nocardiasis the absence of granules on direct examination was a striking feature. There are only two reported cases of spontaneous animal infections by *N. brasiliensis*, one in a cat (Ajello *et al.*, 1961) and the other in an armadillo (Gezuele, 1972). No granules were seen in these infections, which had an acute and fatal course. It is possible that as a consequence of the absence of granules the pathogen may be more vulnerable to both the host's immune reactions and to specific therapy.

The differential diagnosis always includes lymphangitic sporotrichosis. Lymphangitic nocardiasis is, however, a more acute process, the initial lesion being less verrucous and more inflammatory; the lymphangitic cord is less evident and the nodules are larger, showing greater infiltration, with a tendency towards earlier suppuration. Microscopic examination and culture permits definitive diagnosis and the institution of proper therapy.

N. brasiliensis was recognized as the aetiological agent of five of the eight previously reported cases of lymphangitic nocardiasis. Guy (1920) was not able specifically to identify his strain, while Baumgarten (1961) claims the pathogen in his case was *N. asteroides*. However, he used only microscopic morphology and acid-fastness as identifying criteria, and thus *N. brasiliensis* cannot be excluded. Moreover, Raich, Casey & Hall (1960) described a case of cutaneous nocardiasis resembling sporotrichosis in a patient with pulmonary sarcoidosis. The organism was classified as *N. asteroides* because of the failure to liquefy gelatin and to coagulate milk.

Gordon (1964) reported a patient with bilateral ulcerated lesions of the legs, from one of which *N. brasiliensis* was isolated. We have not included this case because of the absence of lymphangitis, and because the pathological study failed to show actinomycetic structures.

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Psoriasis exacerbated by hypoparathyroidism with hypocalcæmia

GUNVER RISUM

Department of Dermatology, The Finseninstitute, Copenhagen, Denmark

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SUMMARY

A case of untreated primary hypoparathyroidism with psoriasis vulgaris is reported. On systemic prednisone therapy the patient developed generalized erythroderma, pustular psoriasis of the soles, candidiasis of the mouth, dermatogenic enteropathy and manifest tetany. Correction of the serum calcium improved the severe skin lesions, emphasizing that fluctuations in serum calcium affect psoriasis.

Hypoparathyroidism may be associated with dermatological diseases as diverse as impetigo herpetiformis (Kaposi, 1887; Kyrle, 1926; Friedmann, 1932; Lutz, 1935; Schmidt-La Baume-Mannheim, 1936; Beek, 1951; Ferriman, 1953), eczema (Winterstein, 1935; Plügge, 1938; Dent & Garrets, 1960), exfoliative dermatitis (Lachmann, 1941; Harell-Steinberg *et al.*, 1957), psoriasis (Laymon & Zelickson, 1959; Garrets, 1961; Vickers & Sneddon, 1963; Montgomery, 1964), candidiasis (Thorpe & Handley, 1929; Simpson, 1954; Bronsky *et al.*, 1958; Kenny & Holliday 1964; Clarke, 1969; Nally, 1970), and other lesions (Simpson, 1954). All these diseases improve on treating the hypoparathyroidism, which corrects the serum calcium level.

This case seems to be the first of untreated primary hypoparathyroidism associated with psoriasis vulgaris which on systemic prednisone therapy progressed to erythroderma with pustular psoriasis of the soles, dermatogenic enteropathy, candidiasis of the mouth and manifest tetany, all of which subsided merely on treatment of the hypoparathyroidism, correcting the serum calcium from greatly reduced to normal levels.

CASE REPORT

A man, aged 29, formerly a labourer, had for the past 2 years received a disablement pension because of psoriasis and epilepsy. There was no family history of psoriasis.

Throughout childhood and adolescence repeated episodes of odd sensations in the chest and abdominal pains caused admission to hospital on several occasions, but no signs of organic disease were detected.

Aged 28 (1969) a syncopal attack caused him to fall down the stairs. A fracture was found in the right parietotemporal line necessitating transfer to a neurological department in Denmark. Phenytoin treatment was started because of a suspicion of epilepsy, and has been continued ever since. Periodically he experiences mild sensory disturbances in the hands, feet, lips, and tongue.

Since the age of 18 the patient had suffered from psoriasis, affecting mainly the knees and elbows. From 1968 to 1970 he had been admitted five times to various dermatological departments because of generalized psoriasis. From December 1969 to May 1970 he was treated with methylaminopterin (Methotrexate), but it was discontinued because of nausea and lack of effect.

Clinical findings

He was admitted as an emergency to a medical department in February 1971 with convulsions. He was then transferred to the Department of Dermatology, Finseninstitute, in March 1971 for treatment of erythroderma and pustular psoriasis of the soles. The nails were affected by the psoriasis. The hair was diffusely thinned. General medical examination on admission was essentially normal.

During the first 3 weeks the patient ran a high fever, and had diarrhoea and retention of urine. He had repeated spasms of the tongue, lips and arms, accompanied by precordial pressure and tachypnoea. The erythroderma deteriorated despite topical therapy, and candidiasis developed in the mouth. Treatment with methylaminopterin (Methotrexate) tablets was started, 35 mg once weekly, but without any effect upon the skin. Owing to the fever and his increasingly debilitated general condition, the patient was given antibiotics and systemic steroid therapy. 48 h later the fever had subsided, but the general condition remained poor, and the erythroderma worsened.

After one week on steroids and antibiotics the patient developed typical carpopedal spasms accompanied by sensory disturbances in the tongue and lips with nausea and vomiting. Both Trousseau's and Chvostek's signs were positive. The total serum calcium was 3·9 mg/100 ml. Injection intravenously of calcium laevulate, 0·1 g/ml, to a total of 20 ml, stopped the attack. The total serum calcium rose to 5·4 mg/100 ml.

All other treatments were then withdrawn, and the patient was treated with Calcium Sandoz® tablets, 3–4 g daily, ultraconcentrated calciferol oil, 90,000–135,000 units daily, and a low-phosphate diet, i.e. fat meats and many vegetables. As the serum magnesium was 0·7 mmol/l, serum folic acid 5 μg/l, and serum potassium 2·7 mmol/l, a magnesium chloride mixture was administered, 10 g, three times daily, folic acid tablets 5 mg × 3 daily, and potassium chloride tablets 1·5 g × 3 daily. Emulsifying cream was used on the skin.

In a few days, all the symptoms disappeared. After a month his hair was growing vigorously. A distinct transverse groove was seen on the nails (Beau's line). The candida infection in the mouth subsided. During a transient decrease in serum calcium the erythroderma became worse on the face, but improved again when the serum calcium was restored to normal.

At follow-up 6 months later the patient reported that he was feeling perfectly well on continued treatment with Calcium Sandoz® tablets, 1 g three times daily, ultraconcentrated calciferol oil, 90,000 units daily, magnesium chloride mixture, 10 mg three times daily, and a low-phosphate diet. The phenytoin medication had been discontinued during stay in a neurological department. Minimal psoriatic plaques were seen on the scalp and on one leg. The patient had started light work.

Laboratory findings

ESR 94–12 mm/h. Serum creatinine, normal. Serum electrolytes (see Table 1). Serum folic acid, at lower limit of normal. Serum albumin, reduced. Quantitative fat in stools, slightly elevated. Xylose tolerance, biopsy of small intestine and liver function tests, no abnormality. Intestinal passage, slight mobility disturbances in the small intestine. X-rays of the stomach, chest, the lumbar spine, sacrum, hands, feet, skull and jaws, no abnormalities, apart from lack of teeth. Ophthalmological examination, no abnormalities, in particular no cataract. EEG, at rest and on hyperventilation, slight diffuse abnormality. On photostimulation, severe, diffuse abnormality.

TABLE I. Electrolytes in the serum and the urine

	Serum	Urine
Chloride	94 mmol/l	
Sodium	134 mmol/l	25–145 mmol/24 h
Potassium	2·7 mmol/l	60–170 mmol/24 h
Standard bicarbonate	28·1 mmol/l	
Calcium	3·9 mg/100 ml	0–248 mg/24 h
Phosphorus	6·8 mg/100 ml	9–150 mg/24 h
Magnesium	0·7 mmol/l	

DISCUSSION

The skin disease which most commonly appears to accompany hypoparathyroidism is impetigo herpetiformis. Beek (1957) was able to collect fifty-seven cases of impetigo herpetiformis from the literature covering the period 1930–1951. Out of these fifty-seven patients, twenty-four had hypoparathyroidism.

Candidiasis frequently accompanies hypoparathyroidism, affecting the entire surface of the body, the mucous membranes, and finger and toe nails. Bronsky *et al.* (1958) found candidiasis in 16% of fifty cases having idiopathic hypoparathyroidism.

Hypoparathyroidism in other dermatological diseases has been sporadically reported. A few cases associated with eczema (Winterstein, 1935; Plügge, 1939; Dent & Garrets, 1960) and exfoliative dermatitis (Lachmann, 1941; Harell-Steinberg *et al.*, 1957), cured solely by treatment of the hypoparathyroidism, are on record.

Most patients with hypoparathyroidism do not have psoriasis, but psoriasis is known to have been provoked by the hypocalcaemia of hypoparathyroidism. Laymon & Zelickson, in 1959, described a case of pseudohypoparathyroidism and psoriasiform plaques in the scalp. Garrets (1961) reported a case of psoriasiform lesions and hypoparathyroidism. In 1963, Vickers & Sneddon published two cases in which psoriasis developed in a state of hypoparathyroidism after thyroidectomy for goitre, the skin lesions varying with the fluctuations in serum calcium, a low serum calcium being attended by exacerbation, whereas a return to normal levels of the serum calcium resulted in remission of the psoriasis. Montgomery (1964) reported a similar case.

Whereas hypocalcaemia caused by hypoparathyroidism with psoriasis has seldom been described, episodic hypocalcaemia is by no means rare in psoriasis unaccompanied by hypoparathyroidism, and is observed particularly in the severe, generalized forms of pustular psoriasis (Copeman & Bold, 1965; Baker & Ryan, 1968; Landry & Muller, 1972), and in psoriasis associated with erythroderma (Shuster & Marks, 1970), on account of dermatogenic enteropathy.

The patient now reported had psoriasis and a typical untreated primary hypoparathyroidism in respect of both the nature of the symptoms, their development and subsidence after treatment with calcium and vitamin D, and of the laboratory findings (Sanitoria, 1971).

The mild malabsorption defect must be considered to have been secondary to the erythroderma, as it subsided when the skin disease had been cleared.

The severe hypocalcaemia that caused exacerbation of the psoriasis was due to hypoparathyroidism. Further exacerbating factors included: dermatogenic enteropathy causing steatorrhoea and increased excretion of calcium with the stools; a mild alkalosis, reducing the quantity of ionized calcium; prednisone therapy, which lowers the serum calcium, since corticosteroids reduce parathyroid secretion in patients with hypoparathyroidism (Eliel, Thomson & Chanes, 1965); and prolonged phenytoin treatment, which causes an increased destruction of vitamin D in the liver (Christiansen, Kristensen & Rødbro, 1972).

The practical conclusion of this study is to emphasize the importance of determining the serum calcium levels in patients with psoriatic erythroderma and generalized pustular psoriasis.

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Letterer-Siwe disease in a young adult

R.T.SHAHANI AND A.MILFORD WARD

The University Department of Haematology, Royal Infirmary, Sheffield S6 3DA
and Department of Immunology, Children's Hospital, Sheffield S10 2TH

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SUMMARY

A case of Letterer-Siwe disease occurring in a 16-year-old girl is described. The case fulfills all the accepted diagnostic criteria for the disease save that of age.

Letterer-Siwe disease is a rare disorder of the reticulo-endothelial system, seen almost exclusively in young children. It is recognized by characteristic skin lesions, often resembling those of seborrhoeic dermatitis, generalized lymphadenopathy, hepatosplenomegaly, fever and otitis media. In a few cases there may be involvement of lungs and bones. We describe below what we believe to be the first well documented case of this disease in an adult reported in the English literature.

CASE HISTORY

J.W., a 16-year-old girl, was admitted to hospital complaining of sore throat, fever, nausea and lassitude which had developed over the preceding 2 weeks. She had been treated with broad spectrum antibiotics by her family doctor without effect. On examination, her temperature was 38·9°C, pulse 112 per min, and BP 110/75 mm Hg. There was generalized abdominal tenderness with no specific physical signs. She was moderately anaemic with a mild leukopenia and neutropenia. All other investigations performed were normal except for urine culture which grew *Streptococcus faecalis* sensitive to ampicillin. 6 days after admission she developed a generalized erythematous, maculo-papular and in places pustular, rash, slightly more marked on the scalp and back. A dermatologist diagnosed pustular psoriasis, as the patient had been having treatment for psoriasis since childhood.

The patient then developed moderate splenomegaly. The skin lesions became nodular, and were crusted in some areas. She continued to be toxic and febrile. Repeat blood cultures, Widal and Paul Bunnel tests, Brucella and anti-streptolysin titres and chest X-rays were all negative. Examination of the bone marrow was not diagnostic. She then developed generalized lymphadenopathy, and gradually became deaf. At this stage leukaemic involvement of the skin was suspected, and as a skin biopsy suggested a malignant reticulosis, the patient was transferred to Sheffield with this diagnosis some 4 weeks after her original admission.

On arrival, clinical examination showed a slightly plump young girl who looked ill, with a generalized erythematous, maculo-papular and in a few places nodular and crusting rash. She had

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Correspondence to: Dr. A.Milford Ward.

marked halitosis, the throat was congested and a few necrotic areas were seen in the mouth. Her temperature was 38·9°C, pulse 144 per min and regular, BP 100/60 mm Hg. She had generalized superficial lymphadenopathy and moderate hepatosplenomegaly. Her hearing was poor, but examination of the ears showed no abnormality. A few fundal haemorrhages were seen in both eyes. Haematological investigations were unchanged, save for a thrombocytopenia of 30,000/μl. Bone marrow aspiration showed non-specific toxic changes with no evidence of leukaemia. The blood urea was 42 mg/100 ml, plasma sodium 120 mmol/l, potassium 3·6 mmol/l and bicarbonate 18 mmol/l. Chest X-ray was normal. Urine culture showed a light mixed growth of contaminants only.

The patient was seen by the Senior Dermatologist who made a clinical diagnosis of Letterer-Siwe disease, later to be confirmed by biopsy. She was started on high dose corticosteroid therapy and efforts were made to correct her plasma electrolyte abnormalities. Her general condition continued to deteriorate and she died 6 days after transfer.

Necropsy

Skin. There was a marked centripetal brownish maculo-papular rash with some crusting, more marked on face and trunk but with some extension to the limbs (Fig. 1). There were occasional petechiae and some bruising, particularly over the arms.

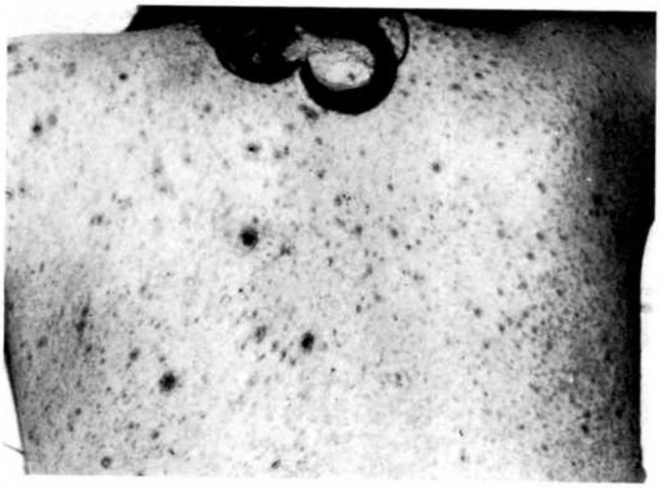

FIGURE 1. Extensive maculopapular rash over the trunk and back.

Lymphoreticular system. There was marked generalized lymphadenopathy. Pale, discrete lymph nodes showed considerable areas of haemorrhage and necrosis. The spleen was grossly enlarged (1000 g) but of normal contour; the cut surface was dark red and soft. Follicular arrangement was obliterated by an infiltrate which appeared both nodular and diffuse. The liver showed some increase in portal markings although the overall size was not markedly increased (2020 g). There was a diffusely nodular infiltrate throughout the lungs, maximal in the subpleural and hilar zones.

Skeletal system. No destructive bone lesions were seen.

Histopathology. The skin lesions showed extensive histiocytic proliferation in the form of focal aggregates arranged mainly in the upper dermis, but also extending around skin appendages. There

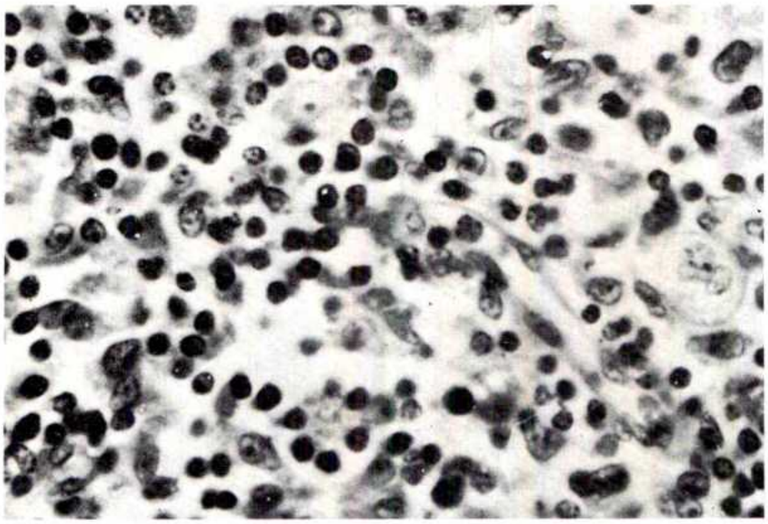

FIGURE 2. Lymph node extensively infiltrated by differentiated histiocytes, replacing the normal lymphoid population (H & E, × 80).

was extension of the infiltrate into the epidermis to give ulceration and some secondary inflammatory features.

The lymph nodes (Fig. 2) and spleen showed extreme histiocytic proliferation with a relatively low mitotic rate. The normal architecture was preserved, although there was severe depletion of lymphoid elements. Haemorrhage and necrosis were common features. The bone marrow was largely replaced by differentiated histiocytes, but there was some continued leukopoiesis and erythropoiesis. The middle ear showed bilateral acute otitis media. The lungs showed extreme congestion with focal collections of histiocytes in relation to the sub-pleural and peribronchial lymphatics.

DISCUSSION

Letterer-Siwe disease in its classical presentation is seen almost exclusively in children. So much is this so that Doede & Rappaport (1967), in a review of ninety-six adequately documented cases of this disease in world literature, made an age of less than 3 years one of their diagnostic criteria.

In an extensive review of the English literature we found only two reports which seem to come close to the criteria for the diagnosis of Letterer-Siwe disease in adults (Dennis & Rosahn, 1951; Schulz, Hamilton & Nay, 1957). The first was diagnosed at necropsy and the features were more those of Hand–Schüller–Christian disease, whilst the second more closely approximated eosinophilic granuloma. Lichtenstein (1953) stated that he had seen several cases in adults but gave no details as the cases had never been fully investigated or followed to their termination. The same was true of Enriquez *et al.* (1967), who in a review of 117 seen at the Mayo Clinic reported four cases in adults.

Scott & Robb Smith (1939) described a few cases of what they called histiocytic medullary reticulosis and felt that the clinical course and presentation of these cases corresponded to Letterer-Siwe disease in adults. They stressed, however, the active phagocytic activity of the medullary histiocytes as a major feature of histiocytic medullary reticulosis, whereas Rappaport (1966) stated that, although phagocytosis may be seen, it is not a prominent feature in Letterer-Siwe disease. It was certainly not striking in the present case. Many instances of atypical lymphoma in adults have since been reported by various authors and mistakenly labelled as cases of Letterer-Siwe disease without fulfilling the accepted diagnostic criteria, diagnosis being based on doubtful histology rather than on clinical presentation.

ACKNOWLEDGMENTS

We would like to thank Professor E.K.Blackburn and Dr I.B.Sneddon for their help and encouragement in the preparation of this report.

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Correspondence

RETINOIC ACID VERSUS PLACEBO IN LINEAR VERRUCOSE NAEVI, SCALY, LICHENIFIED ECZEMA, AND VERRUCAE PLANTARIS

SIR, Vitamin A and retinoic acid seem to influence skin conditions in which the main histological change is epidermal dyskeratosis. Retinoic acid being less toxic than vitamin A could be an therapeutic advance in dermatology, but its value is limited as severe irritant effects may develop in some cases. Steigleder (1971) and McLaughlin (1972) used retinoic acid topically in molluscum contagiosum and in plantar warts and it has been reported that both conditions improved with this form of therapy.

For my investigations twenty patients were selected; verrucose naevi (five cases), scaly, lichenified eczema (six cases), and plantar warts (nine cases). All patients with linear varrucose naevi and three patients suffering from scaly, lichenified eczema were treated with retinoic acid 0.1% in petroleum jelly once daily or every second to third day. In three other patients with scaly, lichenified eczema, retinoic acid was administered in 16 mg doses in hard gelatine capsules once or twice daily. Controlled, unilateral trails with retinoic acid ointment versus placebo were carried out in eight patients. In plantar warts the active preparation and placebo were applied to paired warts after removal of the overlying keratosis, once weekly, and the lesions covered with elastoplast bandage. After 2, 3, and 4 weeks of therapy retinoic acid and placebo treated lesions were curetted for histological examination. During therapy and over the next 3 months all the patients were examined weekly.

The lesions of linear verrucose naevi responded to the regular topical application of retinoic acid 0.1% ointment. Side effects, such as inflammation, peeling, and discomfort, could be avoided when the active preparation was used only once every second or third day. The patients undergoing this form of treatment were under continuous observation, one for 16 months. The brownish keratosis disappeared completely within 4 months. No recurrence was noted when the retinoic acid ointment was then regularly applied once weekly, but a new pigmented linear hyperkeratosis was formed about 6 weeks after withdrawal of the maintenance dose.

In patients with scaly, lichenified eczema, treatment with topical or systemic retinoic acid resulted either in no improvements or a deterioration in the dermatosis. Such medication gave rise to discomfort with inflammation, vesiculation, or a papular eruption with severe itching within a few days, necessitating the withdrawal of treatment.

Topically applied retinoic acid 0.1% ointment was likewise unsuitable for treatment of plantar warts. After 2 weeks, maceration with tissue necrosis was noted and this process became more evident the longer treatment was continued. Although some of the superficial lesions together with the necrotic tissue could be easily removed, the deeper warts remained very painful and no significant histological differences were observed in the retinoic acid treated warts compared with placebo. In one patient a deeper lesion became painless, but when the treatment was stopped a mosaic wart developed.

Department of Dermatology
University of Glasgow

S.H.GUNTHER

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OVERCURVATURE OF THE NAILS—AN INHERITED DISORDER

SIR, One nail abnormality which can give considerable discomfort is the entity described by Samman (1972) as overcurvature of the nails. Due to excessive curvature of the nail in its long axis, the edge of the nail plate tends to cut into the lateral nail fold, giving the same effect as an ingrowing toe-nail. The curvature may become so extreme as to give excruciating constant pain from strangulation of the soft tissues.

Samman remarks that excessive curvature of several nails is seen occasionally and appears to be a developmental anomaly. A patient of Irish ancestry who presented recently with this disorder (Fig. 1) gave a history of other members of her family being similarly affected through three generations. She, three sisters and a brother are affected, while four sisters and three brothers have normal nails. Her father, three paternal uncles and her paternal grandfather were also affected. Onset is always in the teens or early twenties. Her children and those of other sibs are too young as yet to show the family tendency. Great toe-nails are always the first and most severely involved, with less deformity of other toe-nails. Finger-nails are minimally affected.

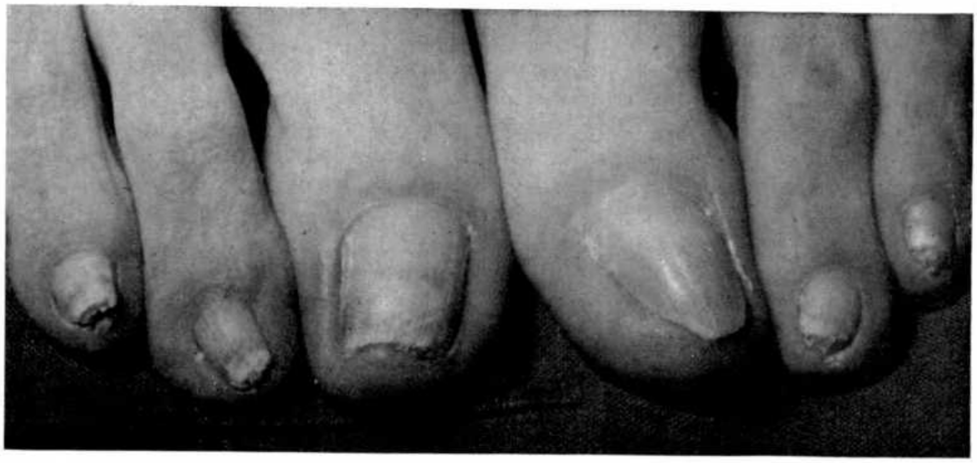

FIGURE 1. Overcurvature of the nails.

With direct transmission through three generations, the condition would appear to be inherited as an autosomal dominant Mendelian characteristic.

Department of Dermatology
Stobhill Hospital
Glasgow G21 3UW

R.S.Chapman

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British Journal of Dermatology (1973) **89**, 319.

Book Reviews

Fotobiología y Dermatología. J.PIÑOL AGUADÉ, J.M.MASCARÓ BALLESTER, J.R.GUIX MELCIOR, J.PUJOL RIBALTA and M.LECHA CARRALERO (1972) Barcelona: Gráficas Marina, S.A. Pp. 282, figures and tables.

The authors deal first with certain fundamental aspects of photobiology as it releates to the skin, discussing the solar spectrum, the significance of action and absorption spectra, the formation and function of melanin, the penetration of light, and the physical and chemical changes which occur following the absorption of light energy. Mention is made of the light sources available for irradiation of the skin, used either with filters or in association with a monochromator, and their relative advantages are discussed. The various procedures are listed which can be applied to the investigation of photosensitivity reactions, such as the determination of minimal erythema dose levels and the technique of photopatch testing.

This introductory part is followed by a detailed description of clinical aspects, dealing first of all with photosensitivity reactions following the presence in the skin of recognizable photoactive chemical substances, such as those resulting from the therapeutic administration of drugs or from a metabolic source as in porphyria, or from contact exposure, as with the salicylanilides and psoralens. The vexed question of photoallergy is reviewed and comparison made with various aspects of the phototoxic reaction. A discussion of the idiopathic photodermatoses follows and deals with the clinical presentation, natural history and action spectra of such conditions as solar urticaria, polymorphic light eruption, summer prurigo, and actinic reticuloid. This review of the present status of the photodermatoses is completed by comment on those conditions, such as psoriasis, eczema and lupus erythematosus, in which exposure to light can play a part in the natural history of the eruption. The book ends with chapters dealing with a study of the cellular effects of ultraviolet irradiation of the skin with particular reference to DNA damage and repair, with UV carcinogenesis, and, lastly, the choice of suitable chemical and physical light screening agents.

The small number of coloured clinical photographs failed to illustrate the characteristics of some of the chosen conditions, and good black and white prints along with drawings illustrating distribution and morphology might well be better. A reasonably adequate bibliography is provided at the end of each chapter. There is no index.

Despite the reviewer's limited knowledge of Spanish, he was able to appreciate that the authors had succeeded in their aim to produce a comprehensive review of the photobiological aspects of skin reactions and had done so in the form of a simple and well planned short book.

W.FRAIN-BELL

Soap Photodermatitis. Photosensitivity to Halogenated Salicylanilides. P.S.HERMAN and W.M.SAMS JR (1972) Springfield, Illinois: Charles C. Thomas, Pp. x + 181, 24 illustrations, 1 colour plate. Price £6.63.

This book stems from the work carried out by Dr Herman for the preparation of a thesis, and as such incorporates a review of the literature and the results of some original work.

The literature review is extensive but could, with advantage, have been shorter by omitting some of the less relevant immunological details, such as those concerning serum 'transfer factor'. In addition, the review is at times not nearly critical enough. The 'sunburn' UVR sensitivity present in some patients with a previous photocontact dermatitis ('persistent light reactors') has been said by Willis & Kligman to be due to the presence of long wave UVR in the source used for the tests. This statement is repeated by the authors on p. 61 and presented as established fact. They have overlooked two separate investigations by Freeman & Knox and by Cripps & Enta where sensitivity to 'sunburn' UVR in these patients has been established using monochromatic radiation, thus excluding long UVR contamination.

Some of the work reported here, e.g. the binding of tetrachlorsalicylanilide (TCSA) to protein, was reported by Jenkins and his colleagues nearly a decade ago.

The authors used the Migration Inhibition Test as an *in vitro* correlate of delayed hypersensitivity in guinea-pigs made photocontact sensitive to TCSA. They found that a factor was released when TCSA sensitized lymphoid cells were exposed to a TCSA–guinea-pig albumin antigen. This factor resulted in marked inhibition of macrophage migration and the authors call this factor MIF (migration inhibition factor). One wonders however whether this factor should really be called 'MIF' in the absence of more specific features, particularly as the authors admit that no attempt was made to identify or purify the inhibiting factor.

There is a useful table listing the antibacterial agents in commercial preparations in America; it is a pity that dermatologists in this country do not have access to such information.

The bibliography in this publication is extensive, but one feels that by careful pruning, the contents could have been presented as a journal article rather than as an expensive book.

COLIN RAMSAY

Diagnosis and Treatment of Venereal Diseases. J.SOLTZ-SZOTS and E.M.KOKOSCHKA (1973) Vienna: W.Maudrich. Pp. 131, 15 figures, 7 tables. Price £4.01.

This is another of a recent series of smaller textbooks on venereology which has appeared in the world literature. There are 115 pages of text. This appears unbalanced, as syphilis which is comparatively rare, and gonorrhoea which though common is a relatively simple disease, take up 85 pages, leaving little for the bulk of conditions which venereologists see and which produce many of the problems. There are curious omissions; thus the very rare herpes urethritis is described but not herpes genitalis which is much more frequent than syphilis in both sexes; this is surprising, as the senior author has published a number of interesting papers on the treatment of *Herpesvirus hominis* infections. Reiter's disease (perhaps the commonest form of arthritis in young men), genital warts, pediculosis pubis, amongst others, are not included. Non-specific urethritis is somewhat dogmatically dealt with: 'bacterial' urethritis is given pride of place and space, followed by trichomonas-, fungal-, mycoplasma- and chlamidial urethritis, giving the impression that these organisms will account for non-gonococcal cases. The sad fact is that even if some of these organisms are responsible for urethritis we are left with a sizeable proportion of cases where no aetiological agent has been identified with any degree of certainty. It is stated that the treatment of chlamidial urethritis should consist of a 6-day course of tetracycline, and in order to prevent re-infection the sexual partner should also be treated; it would be agreeable if the prevention of recurrences were that easy!

There are some irritating mistakes, e.g. TRIC agent does not stand for 'trachoma including conjunctivitis' but 'Trachoma Inclusion Conjunctivitis'; moreover, it is wrongly classified as a virus. The references are not representative and at times are difficult to match up with the text.

It is not easy to characterize the reader for whom this book is best suited. It is clearly not the specialist in venereology or neighbouring disciplines; the medical student and general practitioner will find syphilis and gonorrhoea well covered but will get no guidance about many other important facets of the specialty.

GEORGE CSONKA

Harry's Cosmeticology. 6th edition revised by J.B.WILKINSON (1973) Aylesbury: Leonard Hill. Pp. 824, illustrated. Price £13.50.

The relationship between dermatologists and cosmeticians has not always been an easy one. The study and care of the 'normal abnormalities' of the skin has tended to be regarded by the former as flippant and by the latter as an art rather than a science. And a highly competitive art at that. But much has changed in the last two decades. Cosmeticology has come in from the cold, and dermatology has benefitted from the scientific contributions of workers in research laboratories attached to the larger firms in this field.

They have added greatly to our knowledge of the physical and chemical actions of topical agents and have set high standards of purity and elegance in formulation.

The Principles and Practice of Modern Cosmeticology by Ralph G.Harry was first published in 1940 and rapidly became established as a standard reference book on the subject. The sixth edition, renamed *Harry's Cosmeticology* in deference to this, has been extensively revised by Mr J.B.Wilkinson and several of his colleagues at the Unilever Research Laboratories. They have shown themselves to be very aware of the rapid advances that have been made in recent years in the physiology and biochemistry of the skin and its appendages. In deference to this, the introductory chapters have been completely rewritten (by Dr Wilde). Thirty-seven pages out of some 800 gives him little room to manoeuvre, and at times there is an uneven balance, e.g. ochronosis is quoted as a cause of pigmentation but Addisonian pigmentation finds no mention. There are also some oddities of spelling (or misprints)—'lentigens', 'paniculus' and 'pruritis', for instance, which temper confidence in what is, nevertheless, a well-presented account of the present state of knowledge.

Among the forty-five chapters, many of them new or re-designed, I found those on the hair and hairdressing procedures especially interesting, and the inclusion of a chapter on the problems of the adolescent's skin is commendable. Another, on preservatives and anti-oxidants, gives a balanced appraisal of the scope and function of these agents. The book as a whole is a mine of information, not only on formulae but on the basis on which the formulation rests, and one must congratulate the editorial team for the scientific approach that they have offered in support of this. It is perhaps understandable that in discussing the therapy of established dermatological conditions they should be a little less critical—are topically applied corticosteroids really effective in alopecia areata and are topical antihistamines still to be advocated for allergic disorders ? Dermatologists will make allowances; others may not.

These minor criticisms in no way detract from the excellence of this book, which must become a standard source of reference for all dermatologists. There are several new and welcome features: the references are at last grouped at the end of chapters; there is a list of Trade and Registered names of ingredients used in preparations; and there are a number of excellent plates including EM and scanning EM views.

The volume is well and clearly produced, through the thickness of the paper makes it a little bulky. The jacket design is enigmatic, perhaps, in accord with modern practice, to encourage the passer-by to delve within. He would be well advised to do so.

This is a notable achievement, of which the revising team have every reason to be proud.

D.S.WILKINSON

Biological Impacts of Increased Intensities of Solar Ultraviolet Radiation. A report to the Environmental Studies Board of the National Academy of Sciences and National Academy of Engineering (1973) Washington, D.C. Pp. 47. No price given.

We on Earth are protected from much of the ultraviolet radiation (UVR) from the sun by ozone in the atmosphere. It has been suggested that nitrogen oxides (NO) from the exhausts of supersonic aircraft might cause a significant reduction in the amount of ozone and lead to us receiving increased amounts of UVR.

This report details some of the deleterious effects on animal and plant life which may occur should such a reduction take place. We are told that a 5% reduction in the ozone would lead to '8000 extra cases of skin cancer a year in the white population of the United States'. How such a figure is obtained is uncertain when the dose-response relationships and action spectrum for UV-induced skin cancer in man are quite unknown. There is no doubt that the recommendations made in this report are valid and important, i.e. that much more information on the intensity and distribution of solar UVR is required, together with more knowledge of the biological affects of such radiation.

What is a little worrying is that the report, issued as it is by such august bodies, may well be based on a false premise. To produce the same amounts of NO as were produced by nuclear explosions over the past 20 years, would require very large numbers of supersonic aircraft flying for many hours a day. What is particularly interesting, however, is that these nuclear explosions produced no detectable variation over the normal range of changes in total atmospheric ozone (Goldsmith *et al.* (1973) *Nature*, to be published).

Thus, present evidence, experimental and observational, suggests that it is most unlikely that supersonic aircraft flying at the altitude at which Concorde is planned to fly, viz.17 km above sea level, would have any detectable effect on atmosphe ricozone. The rather pessimistic attitude of this report may well be unjustified.

COLIN RAMSAY

Actas del VII Congreso Hispano-Portugués Dermatologia Médico-Quirúrgica. Granada (1969, published 1973) Pp. xl + 1155, illustrated.

The seventh Spanish–Portuguese Congress of Dermatology was held in Granada from 22 to 25 October 1969, under the presidency of Professor Felipe de Dulanto y Escofet. The proceedings of the Congress are now published in a beautifully printed and handsomely produced volume. The long delay in publication is to be regretted and could surely have been reduced by firm editorial discipline, but it does not detract too much from the value of a book, which contains a great deal of interesting material. The main themes were occupational dermatoses, dermatomycoses, lupus erythematosus, and plastic surgery in dermatology, but there were also symposia on adenoacanthoma, physical therapy and topical medication and contemporary venereology, and a round table discussion on the future of dermatology and dermatologists. The individual contributions are too numerous to list. Most of them are excellently documented, and particularly in the fields of occupational dermatoses and mycology have a special interest for workers in other countries, in that the range of experience in Spain and Portugal is somewhat different from that in North West Europe or in most of the United States.

These proceedings should be in the larger dermatological libraries, as much good work in the Iberian peninsula is less well known elsewhere than it deserves to be.

ARTHUR ROOK

The Challenge of Life: Biomedical Progress and Human Values. Roche Anniversary Symposium. *Experientia*: Supplementum 17. M.KUNZ and H.FEHR (Eds.) (1972) Basel and Stuttgart: Birkhäuser Verlag. Price: £4.38.

'Wasn't that a dainty dish to set before the king?'

Hoffmann–La Roche and Co. was celebrating its seventy-fifth birthday at Basel. Eminent men and women from Western Europe and the U.S.A. professing medicine, biology, economics, sociology, philosophy, theology and law had been invited thither to consider the effects of advances in bio-medical knowledge upon the life of man.

Our own familiar world has reached a critical stage of its evolution. Man has emerged as the dominant living force. Our predecessors learnt to use tools (which they also made into weapons) and to grow food. The relaxed pace allowed time for adaptation. The 'discovery' of the scientific method and its relentless pursuit has increased man's power and enlarged his understanding of the mechanism of life to such a degree that he now exerts a potent influence over conception, life and death, that is entirely new and has far reaching consequences. The results of scientific investigation accumulated comparatively slowly at first, but a crisis has been presented by the rising tide of information, its biological importance, and its haphazard and global application. The rate of change and the extent of the changes have quite overpowered man's adaptive mechanism. Matters threaten to get out of hand and it is likely that, unless man uses his native intelligence to control his new capabilities, his species is going to be in for a very disturbed time, if indeed the growing point that he occupies on the tree of evolution proves to be viable.

Taken in this context the symposium generated less of a bang than a whimper. Intelligence and fluency abounded, but on the whole I felt that there was a lack of appreciation of the nature of the challenge to mankind and of the intimate way in which all are involved. Some of the anxieties expressed (or implied) related more to the standing of science in the eyes of mankind than to the condition of mankind itself. 'Perchance he for whom this Bell tolls, may be so ill, as that he knowes not it tolls for him.' On the other hand the size and the age structure of the population, which lends itself to quantification, is very relevant and was vividly presented. The world has not only a much larger population than it has ever known but one that is also increasing fast. Since the expectation of life is lengthening the proportion of elderly people is increasing. If the birth rate falls a decreasing number of young people will have to bear an increasing burden of the elderly. Is it too fanciful to think of a situation in which the young of the world do little more than keep the old alive ? This eventuality is obviously totally unsatisfactory (even if the situation is alleviated by research on the ageing process), and a little thought will reveal a vista of problems, disadvantages, dangers and perhaps disasters in store for humanity, many of which (such as the quality, as opposed to the length, of life) were discussed. Add to these all the other problems that confront modern man, including the impending exhaustion of some of the natural resources of the earth and the poisoning of the environment, then the imagination falters. It is more comfortable to shut one's eyes (which includes succumbing to the inertia that comes from feeling powerless in the hands of 'the administration') and to pretend that it is all a dream.

The symposium reached no clear conclusion about the future. The following is my own interpretation, based on points made by various speakers. Whatever is to be done must be done by men who can look on the truth unafraid. They must be men who are prepared to use all kinds of human knowledge, including science. It is quite clear that to blame science for our predicament, and thus to argue that research should be suppressed or science banned, is not only irrational, but impossible. It is also quite clear that scientific knowledge by itself is not enough. Science has been defined as 'the pursuit of truth', but truth has many facets and science does not comprehend them all. There is an urgent need for a synthesis of present knowledge, requiring co-operation not only between different scientific disciplines, but also between science and other branches of knowledge. Both scientists and non-scientists must acknowledge that truth can be arrived at by different routes, and that the facets of truth revealed are equally valid. If there could be a consensus of opinion among truth seeking men and women as to what road mankind should aim to tread, then there would be hope. Difficult ?—very. Impossible ?—no. But it is so much easier to wait and see. This might be a valid response if man was ignorant; but man has knowledge, and he must use it if he is to be true to himself. Perhaps time is running out.

ALAN LYELL

British Journal of Dermatology (1973) **89**, 325.

News and Notices

Royal College of Physicians of London

Dr C.M. Ross, Overseas Member of the British Association of Dermatologists, was recently elected to the Fellowship.

British Association of Dermatologists

Annual Meeting, Cardiff 1973

At the Fifty-third Annual General Meeting, Dr G.B. Mitchell-Heggs was elected to Honorary Membership; Dr Vladimir C. Andreev of Sofia, Dr Anthony N.Domonkos of New York, Professor Giuseppe Moretti of Genoa, Dr William B.Reed of California, and Professor Nils Thyresson of Stockholm were elected to Honorary Foreign Membership. The following were elected to Ordinary Membership: Dr J.J.R. Almeyda of Enfield, Dr D.B. Brookes of North Lancaster and South Westmorland, Dr J.L. Burton of Bristol, Dr R.P.R. Dawber of Stoke-on-Trent, Dr J.A. Cotterill of Leeds, Dr J.J. Cream of Croydon, Dr K.A. Grice of Watford and Hemel Hempstead, Dr C. Hodgson of Birmingham, Dr M. McKelvie of Slough and Northwood, Dr R.M. MacKie of Glasgow, Dr J.A. Savin of Edinburgh, Dr J.L. Verbov of Liverpool and Dr J.E.W. White of Southampton. Dr Amal K. Kurban of Beirut and Dr R.I. Patel of Nairobi were elected to Overseas Membership.

Postgraduate Medical School, University of Cambridge

The annual introductory Course on 'The Biology of Skin' will be held in Churchill College from 10 to 14 December 1973. Applications should be sent to J.W. Woodcock, M.A., Secretary, Cambridge University Medical School, Hills Road, Cambridge.

Dermatological Society of Singapore

The Dermatological Society of Singapore plans to hold from 7 to 9 May 1974 a Conference of Dermatologists in the South East Asian Region, including Australia and India. The provisional programme is as follows:—(1) Position papers, (a) Organization of Dermatological Services in the various countries, (b) Disease pattern; (2) Leprosy; (3) Mycology; (4) Contact Dermatitis, Photodermatitis; (5) Parasitic Skin Infections; (6) Miscellaneous.

Enquiries may be addressed to Dr V.S. Rajan, Middle Road Hospital, Singapore 7.

APPOINTMENTS

The United Leeds Hospitals

Dr John Anthony Cotterill has been appointed Consultant Dermatologist to the General Infirmary at Leeds. Dr Cotterill qualified B.Sc., with first class honours in physiology, from the University of Durham in 1960. He obtained his M.B., B.S., from the University of Durham in 1963, his M.R.C.P. in 1969, and his M.D. from the University of Newcastle-upon-Tyne in 1972. After a very distinguished undergraduate career, he became House Physician in the Royal Victoria Infirmary, Newcastle-upon-Tyne; he then held posts in medicine in Ipswich and Doncaster before going back to the Leeds General Infirmary as a Senior Registrar in Dermatology. He has carried out extensive investigative work on sebaceous function and on the biochemistry of acne vulgaris.

University of Wales

Dr Ronald Marks has been appointed Senior Lecturer in Dermatology, University of Wales. Dr Marks was educated at St Marylebone Grammar School and Guy's Hospital, London. He obtained B.Sc.(Hons.) in Physiology in 1955 and qualified M.B., B.S. London in 1959. From 1960 to 1965 he served in the R.A.M.C.; he became a specialist in dermatology in 1963 and M.R.C.P. (London) in 1964. From 1965 he worked at St George's Hospital and St John's Hospital. In 1971 he was appointed Research Fellow in the Institute of Dermatology and Honorary Consultant to St John's Hospital. In 1972 he became Consultant Dermatologist to his hospital.

He has published many papers on histopathology and investigative dermatology and has recently been particularly interested in the experimental study of the epidermal response to injury.

Inverness

Dr McCallum has been appointed Consultant Dermatologist at Inverness (Northern Regional Hospital Board, Scotland).

Dr D.I. McCallum graduated M.B.,Ch.B. at Edinburgh University in 1938, then held junior medical posts at Jersey General Hospital and at Edinburgh Royal Infirmary, and in December 1939 joined the R.A.M.C., where he served with a Casualty Clearing Station for 1 year and with Field Ambulances for the subsequent 5 years. He was awarded the Military Cross.

In 1946 he took the Diploma in Public Health, and then continued his studies in the Department of Dermatology at the Royal Infirmary, Edinburgh, under Professor Percival. He was Clinical Tutor in that Department from 1948 to 1951, and was then appointed Consultant Dermatologist to the Nottingham–Newark group of hospitals.

His publications include a study of the histopathogenesis of eczema, dermatological manifestations of giant cell arteritis and of Crohn's disease (jointly with Dr P.D.C. Kinmont), talc granuloma of the umbilicus, and dermatological complications of peripheral vascular disease.

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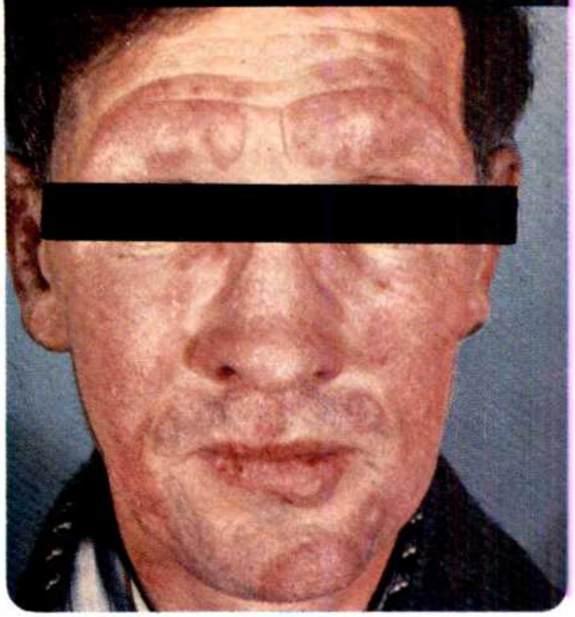
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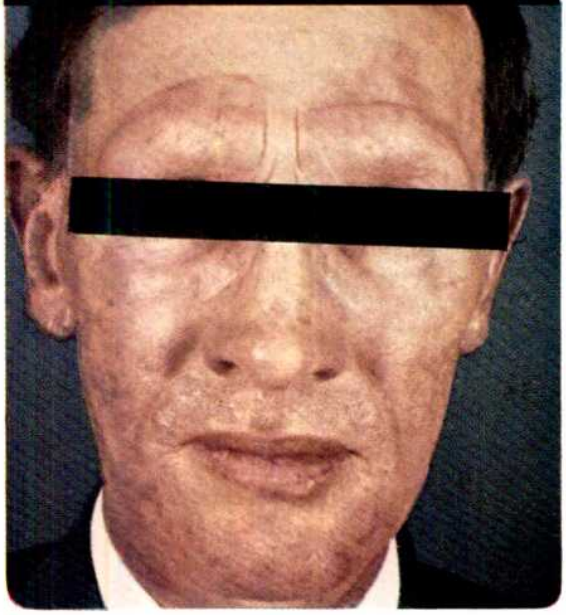
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A Guide to Drug Eruptions

by W. BRUINSMA, *Ministry of Public Health and Environmental Hygiene, Leidschendam*, The Netherlands.

1973. viii + 103 pages. Dfl. 30.00 (about US$ 10.55) Paperback. ISBN 90-219-2075-1

This guide, written specifically for the dermatologist, will be a valuable source of information on the location of harmful drugs. Clinicans, practising dermatologists and research workers in the pharmaceutical industry will welcome this volume in which they will find comprehensive and up to date information.

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REVIEW:

"Dr. Bruinsma's attractively designed volume presents a convenient short practical guide to drug eruptions which compresses into a hundred pages a large amount of essential, well documented and up to date information. . . As now drugs are introduced in such numbers, guides like this are an invaluable aid to any dermatologist in practice. Dr. Bruinsma is to be congratulated on achieving that degree of clarity and simplicity which are the fruit of scholarship and of tedious labour. His book deserves a wide circulation, and it is to be hoped that its success will encourage him to provide revised editions at regular intervals . . ."

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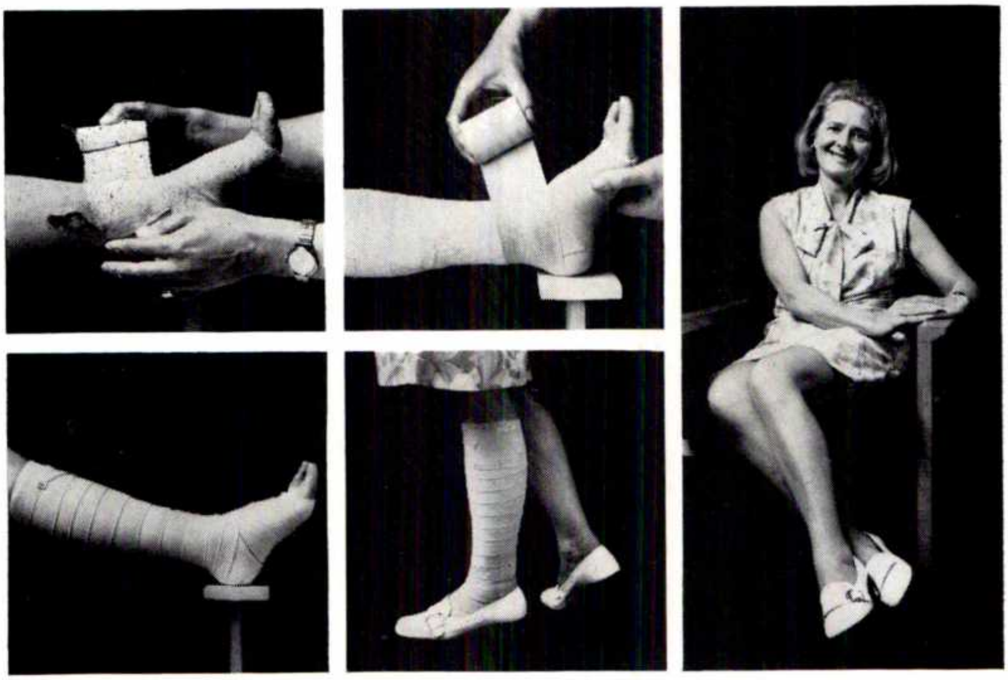

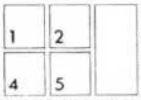

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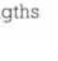

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Manuscripts should be sent to the Editor, Dr A. J. Rook, Old Hall, Steeple Bumpstead, Haverhill, Suffolk. They should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. If, in addition to the original typewritten copy, a duplicate copy, complete with figures, tables and references, is submitted, this will frequently speed publication. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention; new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title unusual abbreviations should be identified; in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	percent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hour(s)	h	milliequivalent	mmol		

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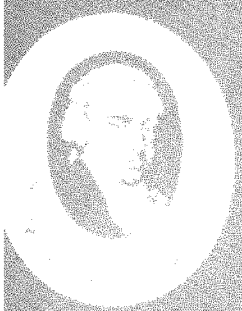

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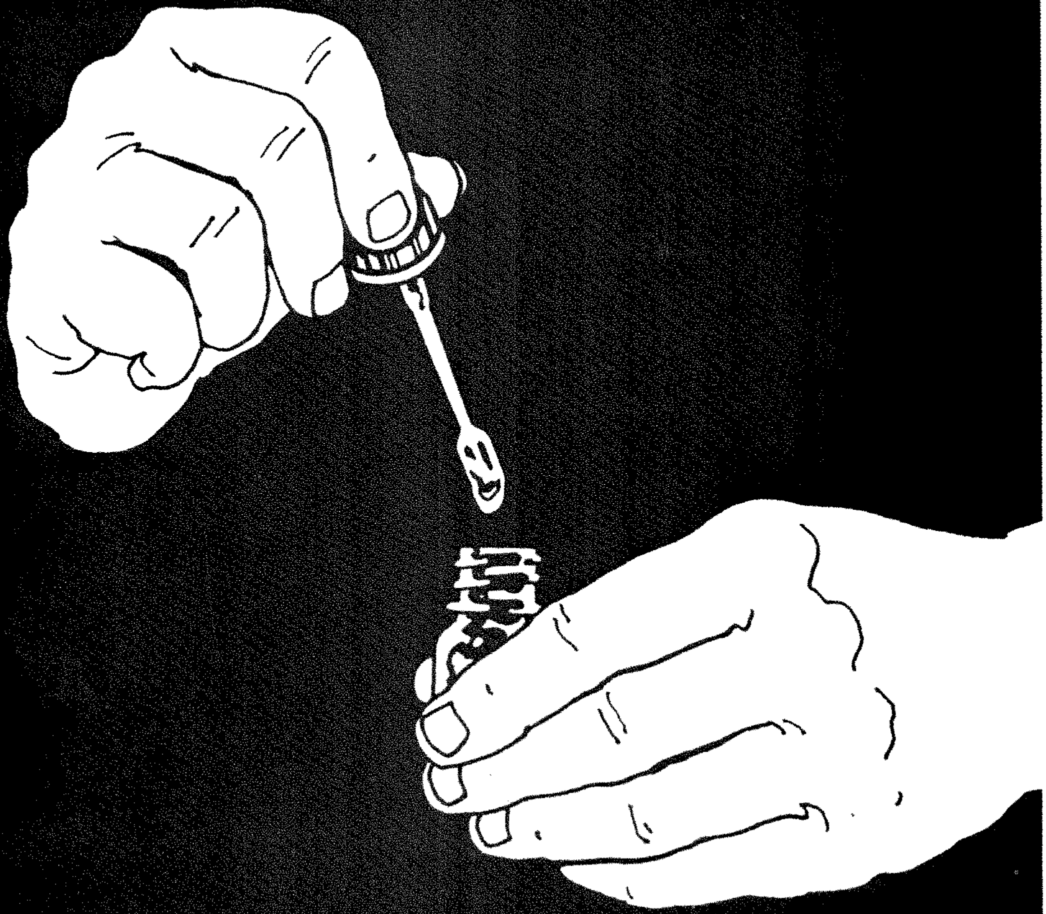

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LEPROSY: Diagnosis and Management (2nd Ed.) *by* **Harry L. Arnold, Jr.** *Univ. of Hawaii School of Medicine, Honolulu, Hawaii, and* **Paul Fasal**, *Leprosy Service, U. S. Public Health Service Hospital, San Francisco.* Provides basics of etiology, epidemiology, classification, diagnosis and treatment. Common misconceptions of infectivity and transmissibility are dispelled. Special emphasis is placed on diagnostic criteria, selection of biopsy site and biopsy technique. Drugs and a dose schedule for treatment are outlined. *'73, 108 pp., 68 il. (32 in full color), 3 tables, $14.75*

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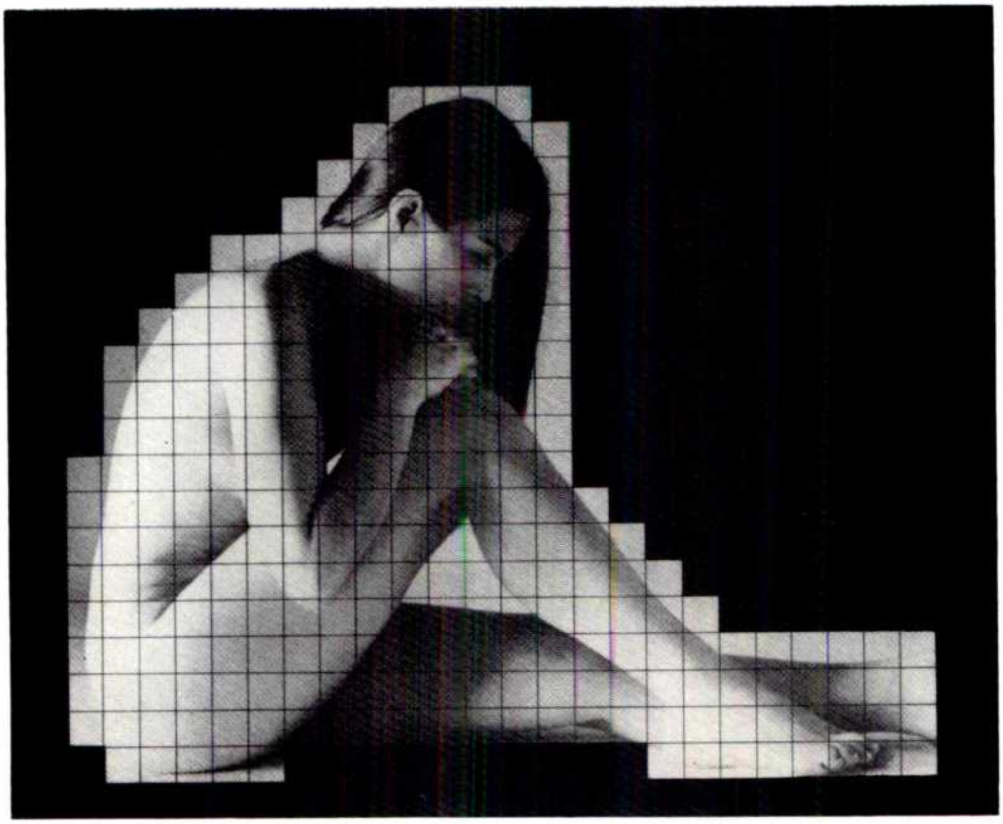

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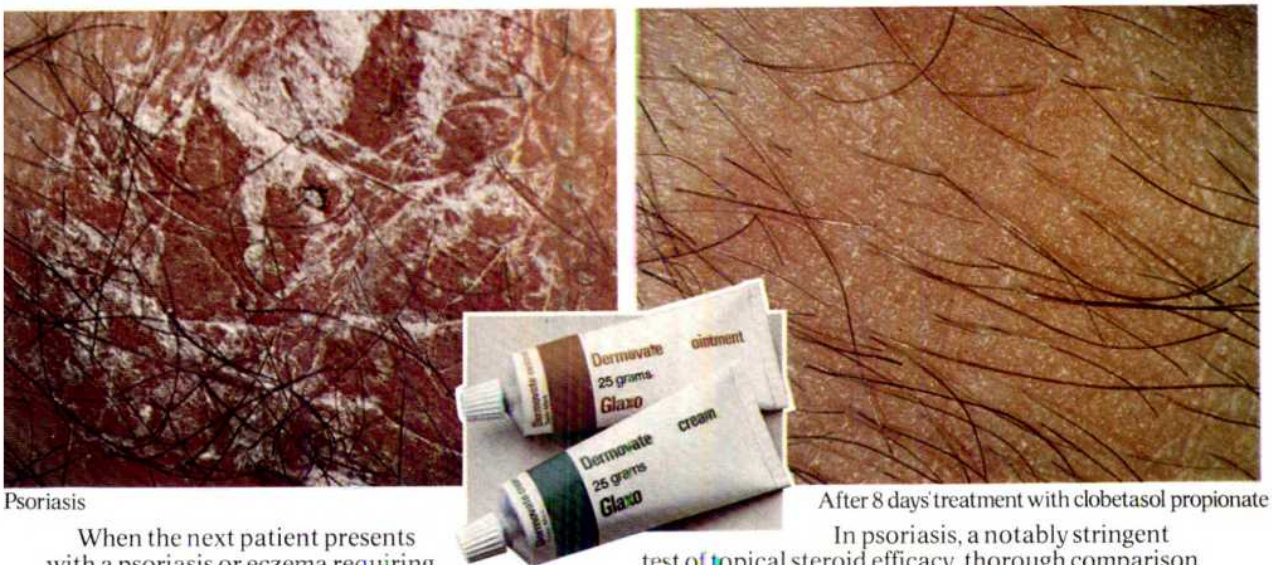

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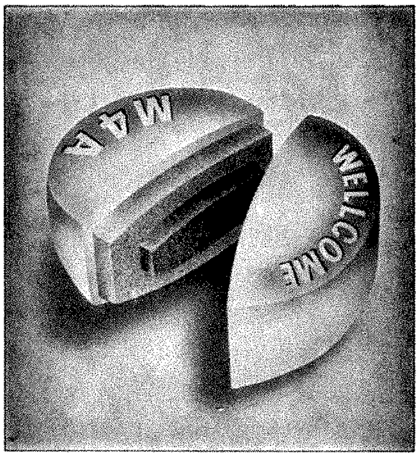

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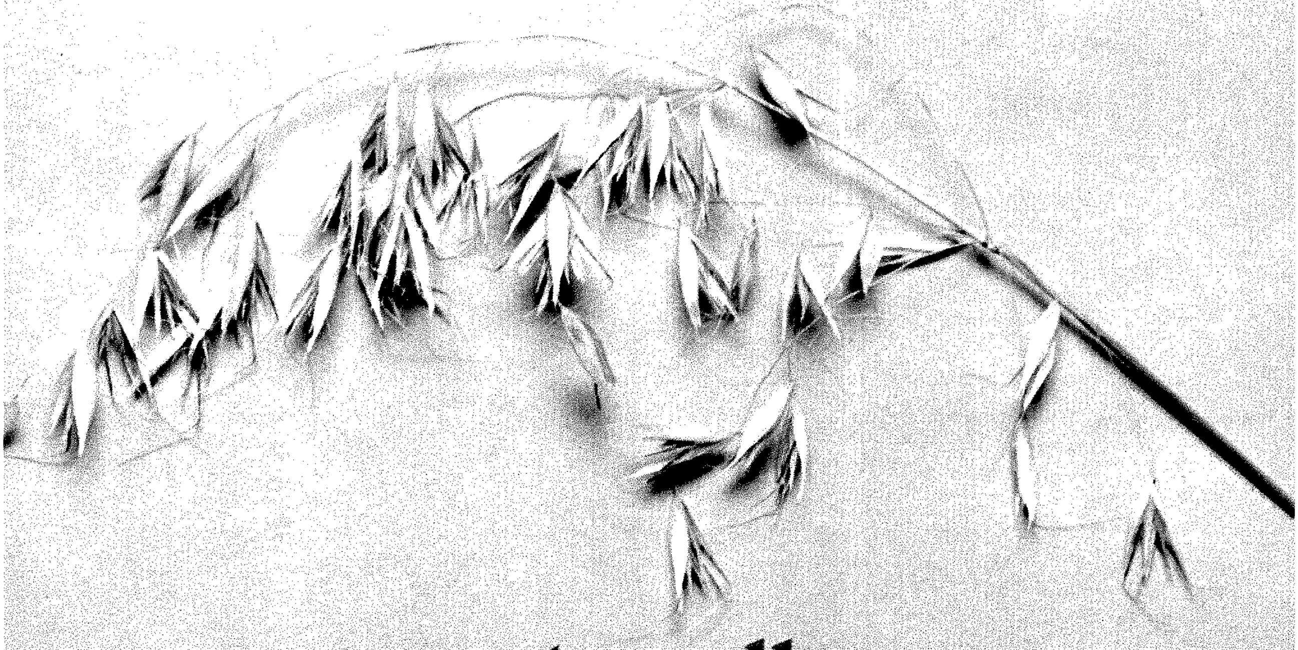

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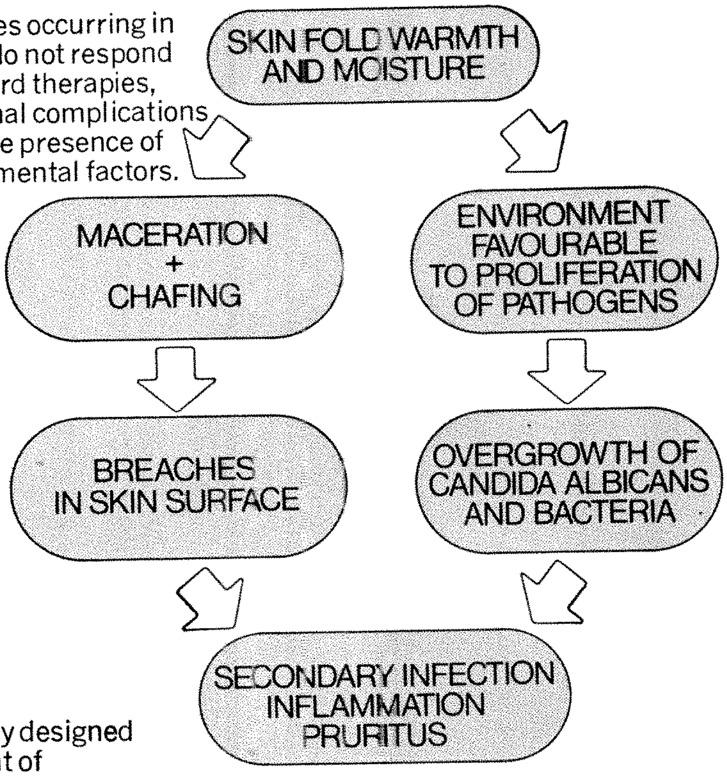
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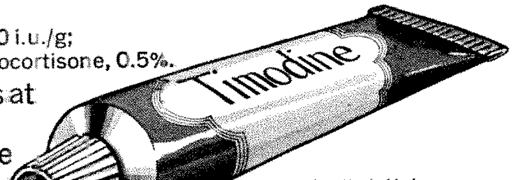

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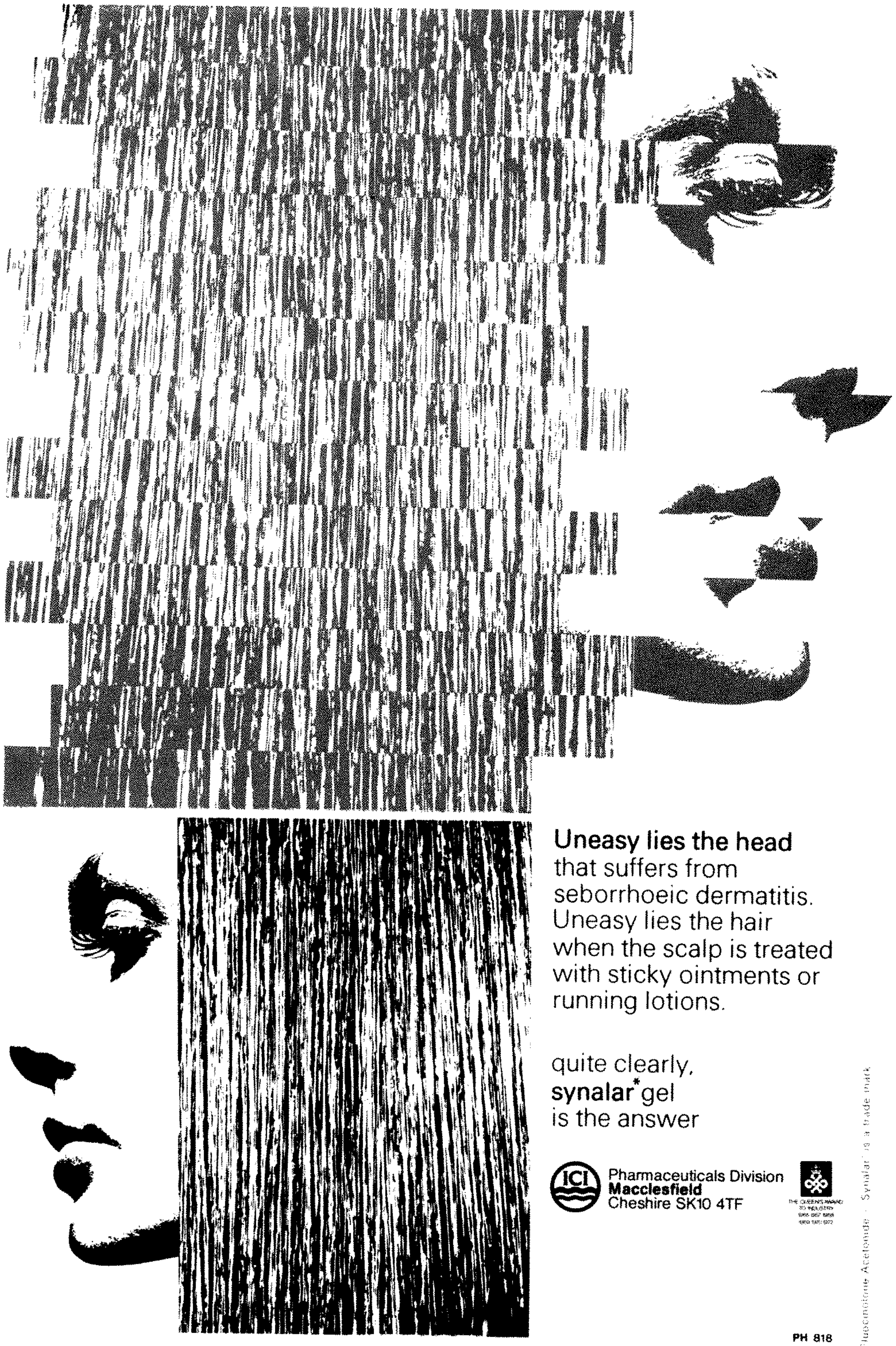

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British Journal of Dermatology (1973) **89**, 327.

Clinical and Laboratory Investigations

Initial events in psoriasis: quantitative assessment*

E. CHRISTOPHERS, R. PARZEFALL AND O. BRAUN-FALCO

Dermatologische Klinik und Poliklinik, Frauentobstr. 9, University of München, Germany

Accepted for publication 30 March 1973

SUMMARY

Seven ^{3}H-TdR-labelled early psoriatic lesions were quantitatively evaluated after serial sectioning and autoradiography. Epidermal thickness was measured planimetrically, whereas labelled cells as well as the number of nuclei present in the dermis were counted.

Marked differences in the number of labelled cells as well as in their grain density were observed. These differences correlated with the density of the dermal infiltrate. It is concluded that psoriatic epidermal hyperplasia possibly develops as a response to antecedent and persisting events in the dermis.

Various techniques have been used to investigate the changes in psoriatic skin. However, as yet no answer can be given as to the question: in which portion of the skin does the process start? Histopathological textbooks nearly always refer to fully developed psoriasis. It is known, however, that the skin lesions start as minute, reddish, slightly raised lesions, which soon begin to scale and become larger by lateral growth. In a previous investigation, histological examination revealed the presence of an intense inflammatory reaction in the upper dermis with only minor degrees of parakeratosis and loss of the granular layer in the overlying epidermis (Christophers, 1972).

In the present study 'initial psoriatic lesions' (IPL) were analyzed quantitatively. The results indicate that the interaction of dermis and epidermis is of importance in the study of this disease. It is suggested that epidermal hyperplasia may develop as a response to antecedent events in the dermis.

MATERIALS AND METHODS

The skin of seven patients with active untreated psoriasis was investigated. All patients were adults with a long history of psoriasis. Large areas of their body were covered with psoriatic lesions and all had developed new lesions within the last 2 weeks. Excisional biopsies were taken of pin-sized lesions characterized by marked reddening and only slight scaling. They were either macules or slightly raised papules.

40 min before the excisions, tritiated thymidine (^{3}H-TdR) (5 μCi dissolved in 0·1 ml saline) (Buchler-Braunschweig, specific activity 5 Ci/mM) was injected intradermally beneath the lesions. The specimens were fixed in neutral buffered formalin, serially sectioned throughout the entire lesions and autoradiographed (Kodak AR 10, 3 weeks' exposure at −20°C). They were stained with haematoxylin after photographic processing.

For quantification, four successive sections demonstrating the most profound alterations were chosen.

---

* Presented at the XIV International Congress of Dermatology, Venice, Italy.

Epidermal thickness was determined planimetrically as previously described (Christophers & Braun-Falco, 1970b) (Leitz Prado slide projector, magnification 300 times normal, Ott-planimeter). Epidermal surface length (ESL) was measured by a map roller (curvimeter) on the prepared paper drawings. The number of ^{3}H-TdR-labelled epidermal cells was determined per ESL, under the microscope. Cells with more than four grains above the generally low background (between one and two grains per cell) were considered labelled. Since in the autoradiographs it was not possible to identify exactly the type of cells present in the dermis, all dermal nuclei were counted in correlation to ESL except adnexal epithelia. The dermal area under investigation extended 200 µm deep into the dermis, starting from the basement membrane.

RESULTS

The histological features of the initial psoriatic lesions (IPL) consisted of massive papillary oedema, dilated capillaries and an inflammatory infiltrate. The epidermis showed some thickening, which never resembled typical psoriatic acanthosis. Parakeratosis was present in most of the specimens, usually immediately above the most severe tissue alterations in the dermis. It was accompanied by loss of the granular layer.

HISTOMETRICAL DATA

In all seven specimens which were quantitatively evaluated (Figs. 1–7), highest values for epidermal

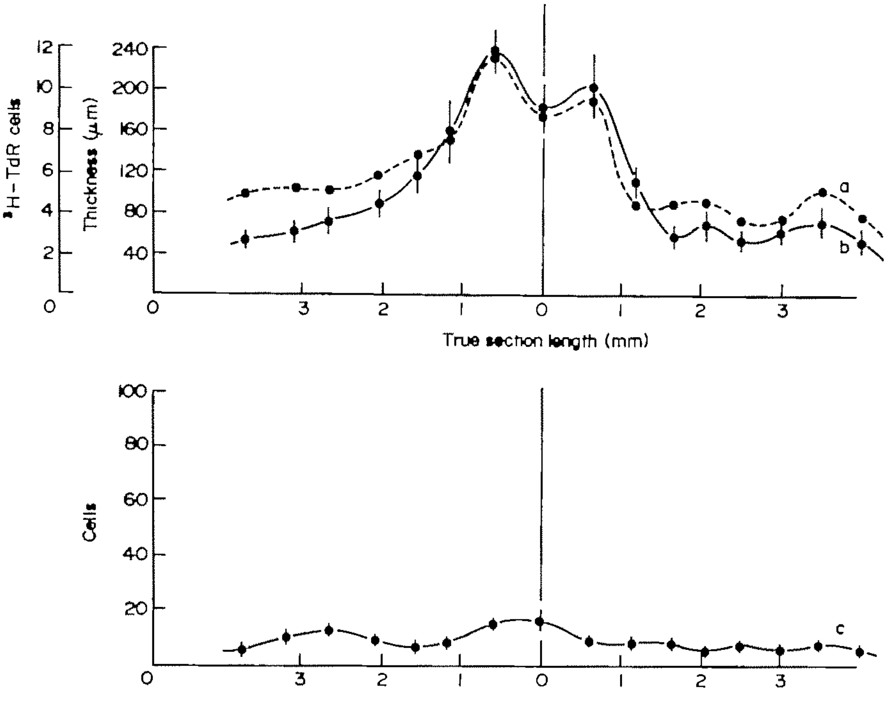


FIGURE 1. Figs 1–7 show the quantitative assessment of seven initial psoriatic lesions per mm true section length (ordinate). a, epidermal thickness in µm; b, number of ^{3}H-TdR-labelled cells calculated per mm epidermal surface length; c, number of dermal nuclei calculated per mm epidermal surface length. All values are the means (vertical bars, standard deviations) of the data obtained from four tissue sections showing the most profound central alterations. F, hair follicles.

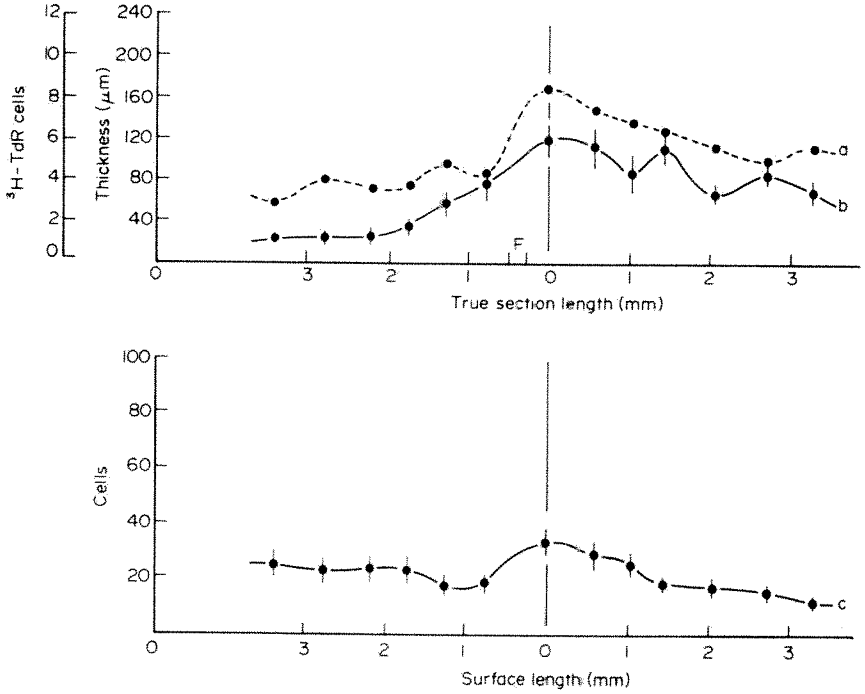
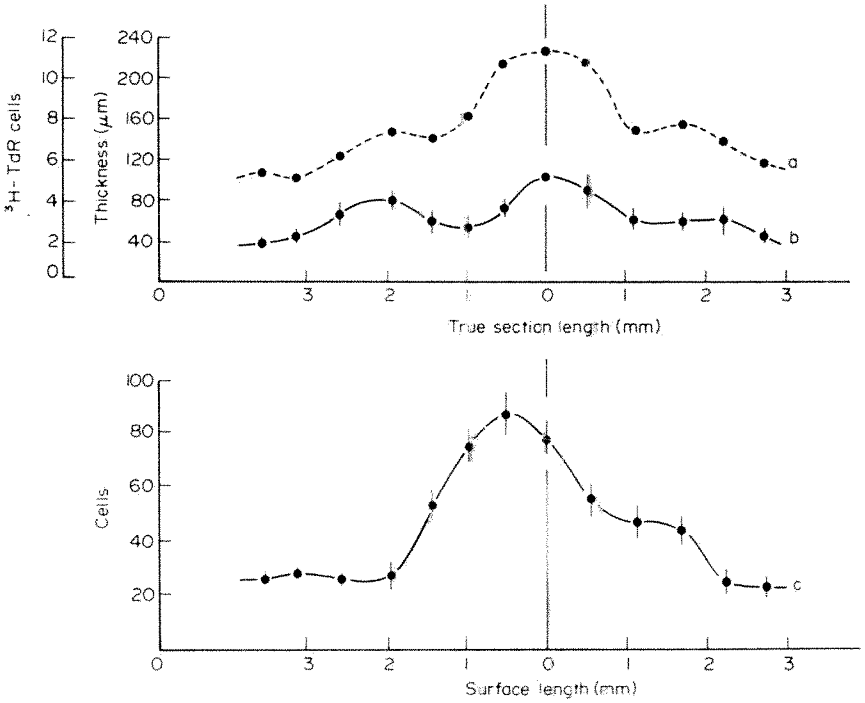


FIGURE 2.



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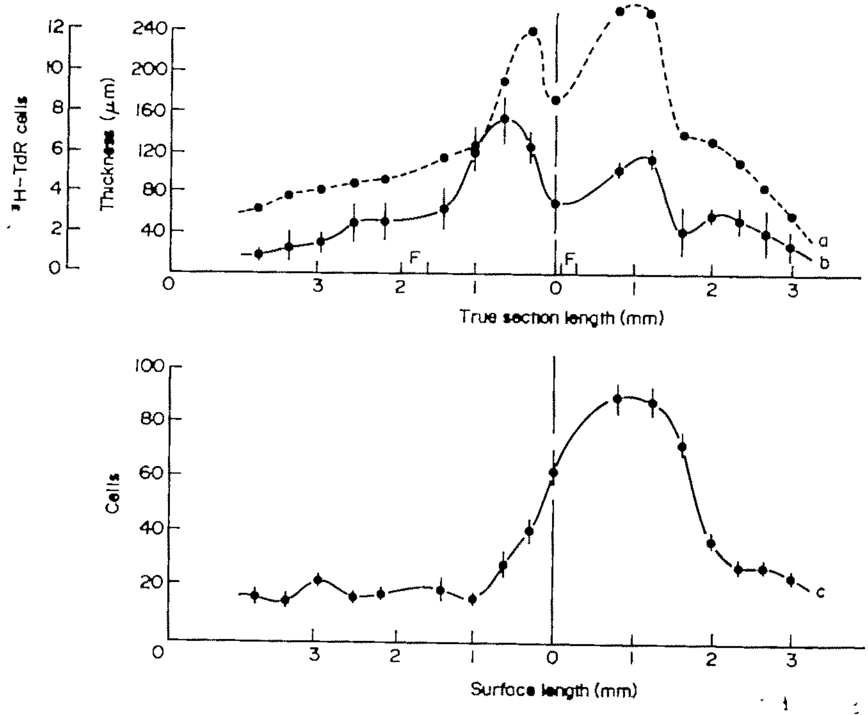

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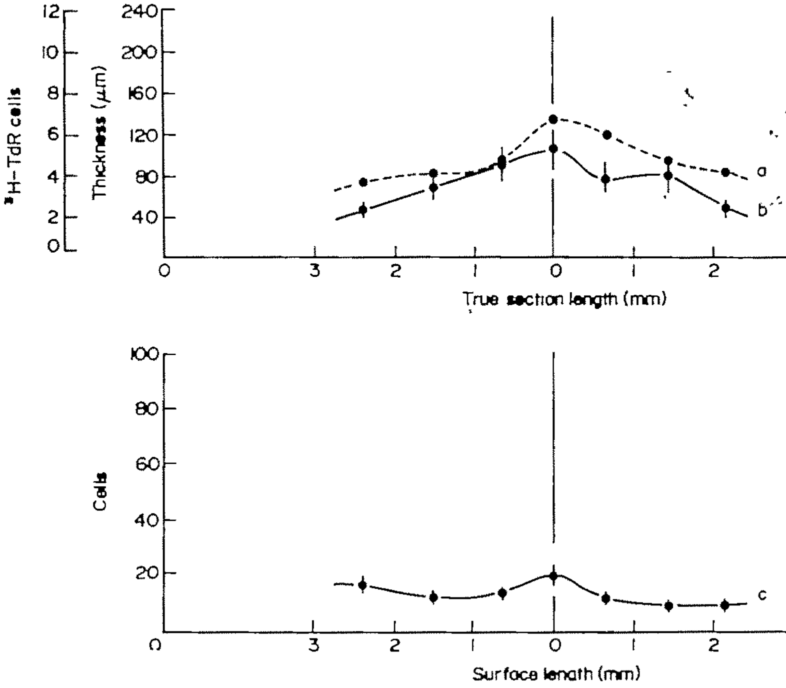

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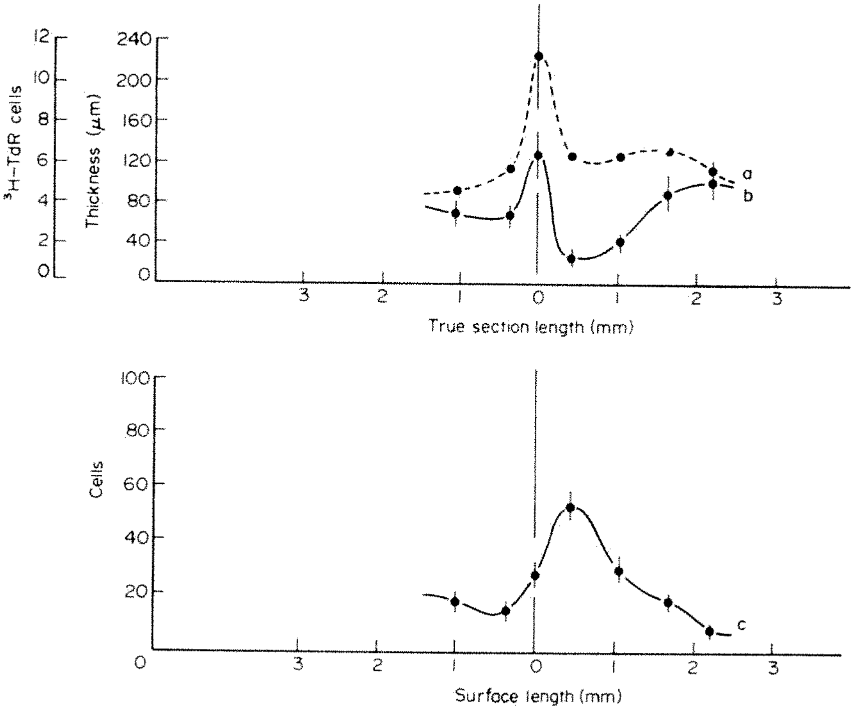

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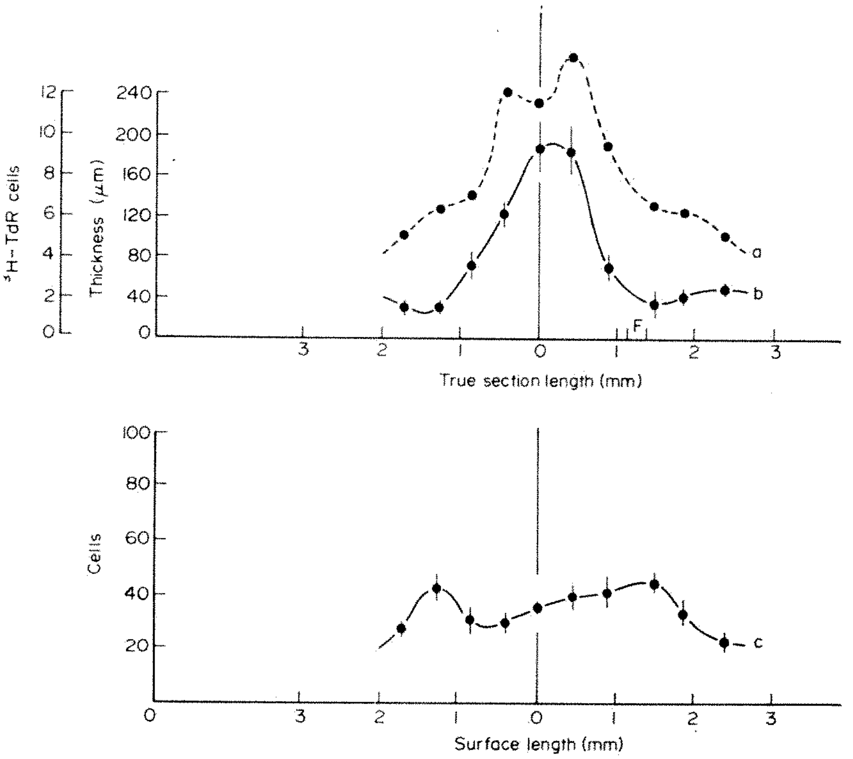

FIGURE 7.

thickness as well as DNA-labelling were found at the centre of the lesions. Maximal epidermal thickness in these regions measured 220–240 μm, which is approximately four times the normal. In one specimen (Fig. 2) the epidermal thickness was only twice normal. Twelve ^{3}H-TdR-labelled epidermal cells per mm epidermal surface length were counted in one biopsy (Fig. 1). This is nearly fifteen times the normal figure. All other specimens showed ^{3}H-TdR-labelling six to seven times the normal.

It was consistently observed that the grain density per epidermal nucleus was lowest in the centre of the lesions, whereas the adjacent epidermal S-phase nuclei (as well as dermal nuclei) showed a high density of silver grains (Fig. 8). Two of the specimens showed an excessive accumulation of inflammatory cells in the centre of the lesions (Figs. 3 and 4). The highest values counted ranged from 80–90 nuclei per mm ESL. In two other lesions the number of dermal nuclei was slightly raised (Figs. 1 and 2), while in the remaining three specimens (Figs. 5, 6 and 7) between 20 and 60 nuclei were counted.

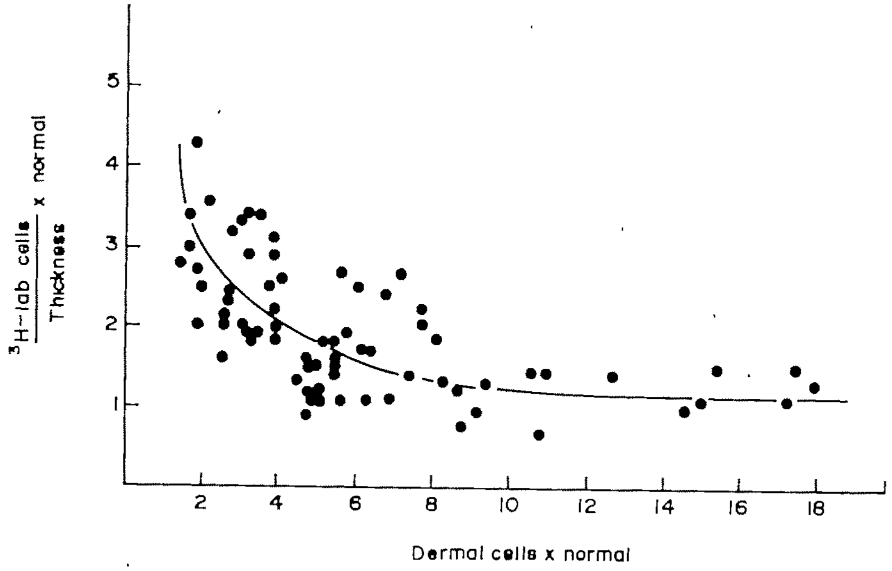

FIGURE 8. Correlation between the number of ^{3}H-TdR-labelled epidermal cells per epidermal thickness and the number of nuclei per epidermal surface length present in the dermis. To allow direct comparison all values are given as the factor of normal.

It was interesting to observe that in the specimens showing the highest density of dermal cellular infiltrate a dissociation between the number of ^{3}H-TdR-labelled epidermal cells and epidermal thickness was present (Figs. 3 and 4). On the other hand, in specimens with a low dermal cell density (Figs. 1 and 2) the proportion of labelled epidermal cells paralleled the curves for epidermal thickness.

For further clarification the epidermal tissue parameters (thickness and ^{3}H-TdR-labelling) were correlated with the changes present in the dermis (mainly 'inflammatory' cells): all data obtained from the biopsy specimens, in which the epidermal thickness had exceeded a baseline value of 80 μm, were computed as a factor of normal. The individual figures presented in Fig. 8, therefore, are taken from the more central portions of each biopsied psoriatic lesion leaving out the margins. Normal values of epidermal ^{3}H-TdR-labelling were taken from the data given by Frost, Weinstein & Van Scott (1966) (0·9 cells per mm ESL).

In ten control specimens obtained from the normal back of adult volunteers the number of dermal nuclei was 5 per mm ESL and the epidermal thickness measured 60 μm.

It can be seen from the calculated curve (Fig. 8) that an inverse correlation exists between the number of labelled epidermal cells/epidermal thickness and the number of dermal nuclei as a factor of normal. This substantiates the previous observation that in specimens with an increasing density of dermal 'inflammatory' cells the number of ^{3}H-TdR-labelled epidermal cells becomes smaller.

DISCUSSION

A main feature of psoriatic skin lesions is epidermal hyperplasia (Braun-Falco, 1963; Van Scott & Ekel, 1963; Christophers & Braun-Falco, 1970b; Cox & Watson, 1972). This is comparable to experimental hyperplasia produced in guinea-pigs by local application of different compounds which stimulate epidermopoiesis (Christophers & Braun-Falco, 1970a).

As a general feature of experimental hyperplasia, the epidermis rapidly becomes normal after the withdrawal of the factor inducing hyperplasia. In psoriasis, on the other hand, it is clinically well known that spontaneous clearing of the skin lesions does occur. The epidermal alterations in psoriasis thus represent an example of benign hyperplasia which shows varying degrees of defective keratinization in addition to increased new cell formation. It is not known by what mechanism this tissue response is initiated and maintained.

In the present study there was a typical histometric profile for all specimens of very early small psoriatic lesions, with epidermal hyperplasia at a maximum in the centre of the lesions.

These bell-shaped curves are obviously found only in early psoriasis, in contrast to larger plaque-like lesions, which show a central plateau (Soltani & Van Scott, 1972). Also, in the central plateau-like portions ^{3}H-TdR-labelling seems to be at maximum, since further stimulation such as cellophane stripping remains without effect there as opposed to the marginal areas (Christophers & Braun-Falco, 1970b). In contrast to fully developed psoriasis, however, marked differences in the number of ^{3}H-TdR-labelled epidermal cells were found in the centre of IPL.

Correlation with the third parameter sought in this study, namely dermal 'inflammatory' cells, shows that with an increasing density of dermal cells the number of DNA-synthesizing cells per epidermal volume becomes smaller (Fig. 8). Furthermore, the intriguing diminution of silver grains in labelled epidermal cells in the centre of IPL contrasted with the heavy labelling of marginal epidermal cells or subepidermal nuclei. Because ^{3}H-TdR-labelling was performed by local injection of the tracer the distribution was not always even, and excluded quantification by counting silver grains. A diminished number of silver grains in fully developed psoriatic lesions as compared to adjacent epidermis was also found by Born & Kalkoff (1969).

The inverse relation between the number of replicating epidermal cells *and* the density of the subepidermal infiltrate is against the concept of psoriasis being exclusively an epidermal disease. These findings suggest rather the possibility that the psoriatic process is initiated in the dermis, by as yet unknown mediators. Such mediators seem to inhibit ^{3}H-TdR-uptake (low grain density) as well as epidermal differentiation in the central portions of IPL. In the marginal areas, however, 'reactive' hyperproliferation leads to epidermal hyperplasia, which in fully developed lesions becomes the prominent feature. Although still speculative, the sequence of events discussed here is favoured by our data.

ACKNOWLEDGMENT

Supported by Deutsche Forschungsgemeinschaft.

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Study of folate in psoriasis: blood levels, intestinal absorption and cutaneous loss

R.TOURAINE, J.REVUZ, J.ZITTOUN*, J.JARRET* AND M.TULLIEZ

Department of Dermatology and *Department of Haematology and Immunology,
H. Mondor Hospital, University of Paris

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SUMMARY

Serum, red cell and squame folate content have been measured in fifty cases of psoriasis with extensive lesions. Reduced serum and red cell folate have been found in 44% of the patients. Folic acid absorption, studied in twenty-six patients, was impaired in four and significantly increased in all the others.

Squame folate content has been found too low to explain the folate deficiency. Malabsorption is a possible hypothesis but it occurs too rarely (four cases). The most likely explanation of folate deficiency seems to be an increased utilization of folate by the skin epidermal cells as a result of the rapid turnover rate.

The increased absorption found in most cases investigated could be a compensatory mechanism to equilibrate folate balance.

Folic acid in psoriasis was first studied using the FIGLU test (Knowles, Shuster & Wells, 1963). More accurate methods of folate assay were later developed by assay on the growth of *Lactobacillus casei* and *Streptococcus faecalis,* which clearly demonstrated the serum and red cell folate to be reduced in psoriasis. But there is some debate as to the mechanism of this moderate folate deficiency.

We have studied fifty cases of psoriasis to determine the most likely factors causing the reduced serum folate levels that are often observed in this disease.

MATERIAL AND METHODS

Patients

All the patients suffering from psoriasis were in hospital. They had extensive psoriasis, and were classified into two groups: very extensive psoriasis in which more than 65% of the body surface was involved, and moderately extensive involving 40–65%. Eight of the patients had crippling psoriatic arthritis or Reiter's syndrome with polyarthritis as well as psoriatic skin lesions. We have not included in this category patients with one or two joints involved, but without severe clinical signs.

Measurement of serum folate

A microbiological assay was made using *L. casei* ATCC 7469, which is auxotrophic for folic acid and the different forms of folate. The technique used is a modification of the method described by Herbert

Requests for reprints to Dr J.Revuz, Hopital Henri Mondor, F. 94 010 Creteil.

(1961). A dilute suspension of *L. casei* is added to different dilutions of deproteinized serum and, after incubation for 48 h at 37°C, the growth of the micro-organism is measured by potentiographic titration using dilute alkaline solution (Zittoun & Zittoun, 1970).

Measurement of the red cell folates

The method of Hoffbrand, Newcombe & Mollin (1966) was used. After measuring the haematocrit, the whole blood was diluted 1:10 in water containing 1% ascorbic acid, then further dilutions were made to bring the folate concentration of the specimens into the range of the serum levels and to fall on the linear part of the dose response curve. The diluted blood was then assayed using the same method as for the serum.

Folate absorption test

The technique of Chanarin & Bennet (1962) was used. The patient was pre-saturated with folic acid during the 48 h preceding the test and received a dose of 40 ng/kg of folic acid on the day of the test. Blood samples were taken after 30, 60, 90 and 120 min, and the folate assayed by *L. casei* (sensitive to the oxidized and reduced mono- and diglutamates), and by *Strep. faecalis* ATCC 8081 (sensitive to the same forms of folate, except N5 methyltetrahydro folate, the predominant circulating form).

Measurement of folate in the squames

Samples of squames were collected and stored at −20°C because of the lability of folate in oxygen and air. The folate in squames is partly in the form of pteroylmonoglutamates, but mainly as pteroylpolyglutamates. For this reason the folate concentration was measured after an enzymatic conversion of the pteroylpolyglutamates into mono- and diglutamates.

Preparation of the squames

200 mg of squames were weighed, and homogenized in 10 ml of phosphate buffer pH 6·2 containing 1% ascorbic acid, using an Ultraturax homogenizer. They were kept for 1 h at +4°C and then homogenized again, autoclaved for 5 min at +120°C, then centrifuged. 2 ml of the supernatant was incubated with 0·05 ml chicken pancreas for 16 h at 37°C (20 mg chicken pancreas mixed in 1 ml of water and then centrifuged).

2 ml of the supernatant after enzyme digestion, and 2 ml of untreated squame homogenate were each made up to 10 ml with phosphate buffer pH 6·2 containing 100 mg/100 ml vitamin C, autoclaved for 5 min at 120°C and filtered. The folate was then assayed in the same way as the serum.

RESULTS

Serum folate

The mean serum folate in fifty normal controls was 8·4±2·6 ng/ml. Of fifty psoriatics, twenty-two (44%) were below normal, i.e. less than 5·8 ng/ml. The mean of this group was 4·25 ng/ml, with upper and lower limits of 2·1 and 5·8 ng/ml. Out of the twenty-two patients with reduced folate levels eleven had very extensive, and eleven moderately extensive psoriasis (Fig. 1).

Red cell folate

All the fifty controls had values of above 200 ng/ml; the mean and standard error were not determined due to the wide variation in the normal results. Red cell folate was measured in thirty cases of psoriasis; sixteen of them (44%) were below 200 ng/ml with a mean of 148 ng/ml. Eleven had very extensive psoriasis, and in five it was moderately extensive. In twelve of the thirty-six patients in whom both serum and red cell folate were measured, both were abnormal (Fig. 1).

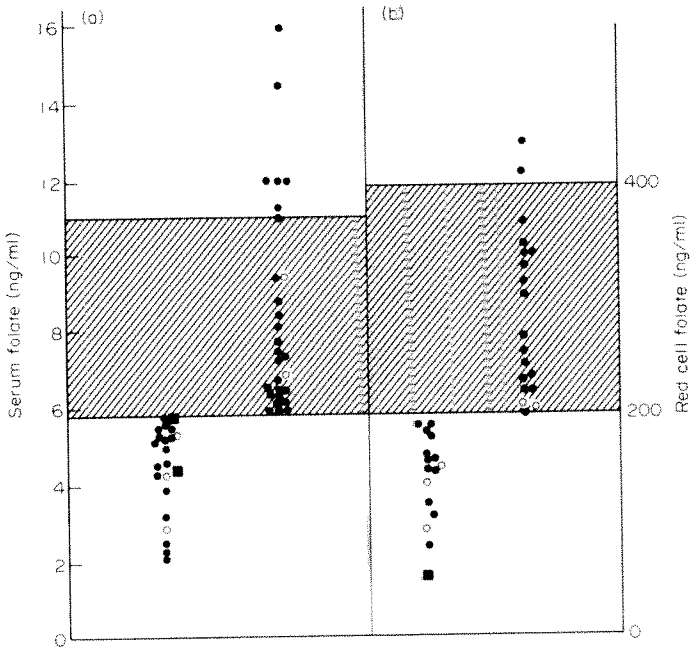

FIGURE 1. (a) Serum folate and (b) red cell folate. Hatched area, normal range; ●, psoriasis; ○, psoriatic arthritis; ■, Reiter's syndrome.

Correlation of serum folate with anaemia

Nine patients had a haemoglobin of less than 11·8 g/100 ml. Among them seven had serum folate levels below normal. Two patients with Reiter's syndrome and two with severe psoriatic arthritis were anaemic. A patient with a hypochromic iron deficient anaemia, probably the result of bleeding, whose serum folate was normal (6·3 ng/ml), was excluded.

Correlation of serum folate with macrocytosis

Fourteen patients had a mean red cell volume greater than 100 μm^3. Three patients were excluded from this study as they had received hydroxyurea or azathioprine. These drugs are liable to cause a considerable increase in the mean erythrocyte volume. Six of the remainder had serum folate below 5·8 ng/ml, and this was normal in the other patients. Three patients were anaemic and two of these three showed low serum folate.

Folate absorption studies

Twenty-six tests were made using the *Strep. faecalis* (Fig. 2) assay and twenty using *L. casei* (Fig. 3). In sixteen patients assays were performed using both micro-organisms. The results suggest a clear difference between two groups of psoriatic patients. In four patients the flat curves indicate a probable absorption deficiency (Table 2); the other patients showed a fairly uniformly raised absorption curve (Tables 1 and 2).

The two groups of patients were separated for more detailed examination of those with elevated absorption curves. The difference between the psoriatic patients and the controls is significant ($P < 0{\cdot}01$) at every time interval during the test when using the *L. casei* to measure folate. However, using the *Strep. faecalis* assay, the difference is significant only at 60 min ($P < 0{\cdot}05$).

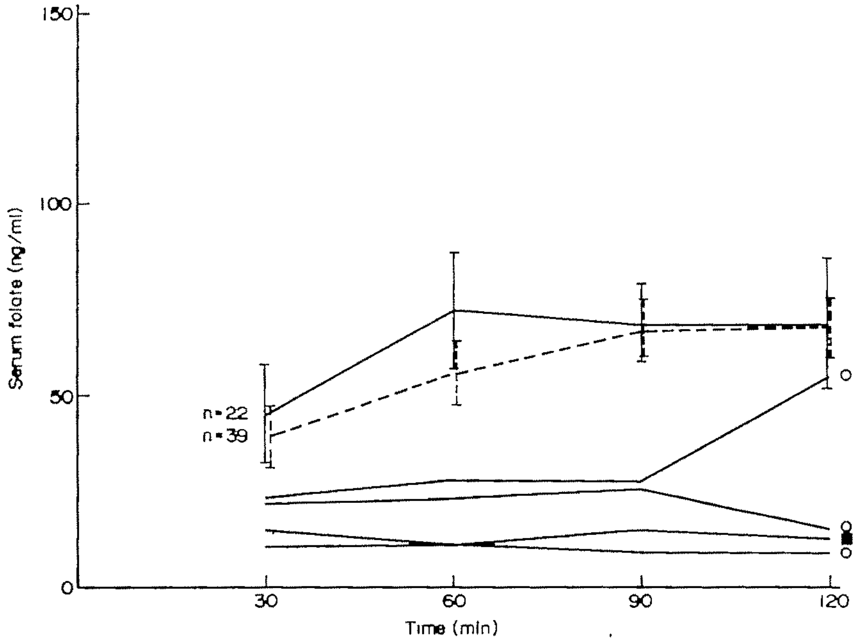

FIGURE 2. Serum folate assay with *Strep. faecalis*. – – – –, normal; ———, psoriasis; ○, psoriatic arthritis; ■, Reiter's syndrome.

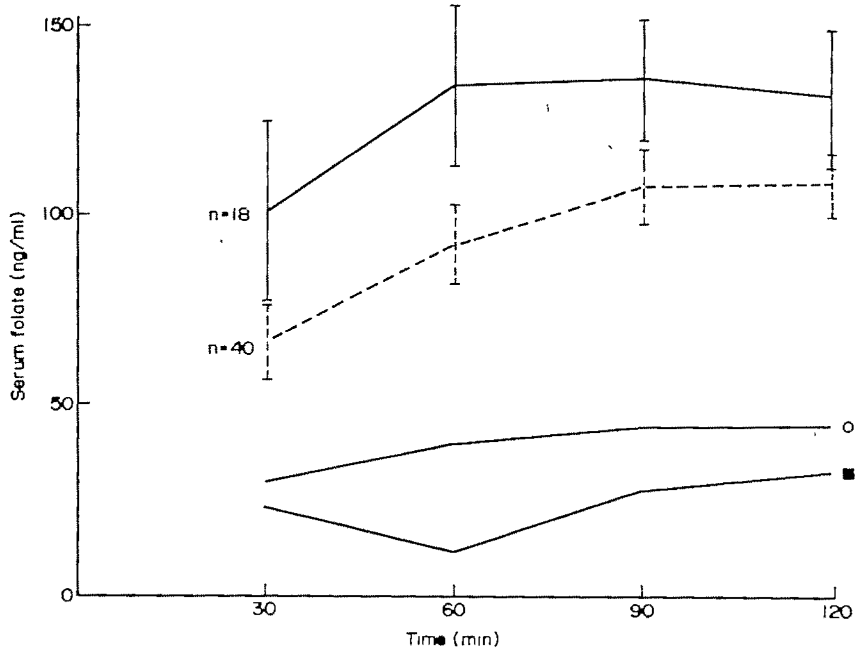

FIGURE 3. Serum folate assay with *L. casei*. – – – –, normal; ———, psoriasis; ○, psoriatic arthritis; ■, Reiter's syndrome.

TABLE 1. Folic acid absorption (*L. casei*)

	Folic acid (ng/ml)			
	30 min	60 min	90 min	120 min
Normal controls ($n_a = 40$)	67±9·9*	92·7±10·7	108±9·8	108·5±8·1
Psoriasis ($n_b = 18$)	101·3±23·6	134·3±21·4	136±16·2	131·3±18·4
t†	3·22	3·92	3·03	2·68

* $\mu+(t\sigma/\sqrt{n})$

† $t = (\mu_a+\mu_b)/\sqrt{[(\sigma^2/n_a)+(\sigma^2/n_b)]}$

TABLE 2. Folic acid absorption (*Strep. faecalis*)

	Folic acid (ng/ml)			
	30 min	60 min	90 min	120 min
Normal controls ($n_a = 39$)	39·4±8·5*	56±8·4	67·3±7·5	67·9±8·2
Psoriasis ($n_b = 22$)	45·2±13·4	72·4±15	68·3±10·2	68·7±17·5
t†	0·76	2·06	0·15	0·01
Patients with flat curves (four)	19	16	16	22

* = $\mu+(t\sigma/\sqrt{n})$

† $t = (\mu_a+\mu_b)/\sqrt{[(\sigma^2/n_a)+(\sigma^2/n_b)]}$

TABLE 3. Squame folate content

Patients	Pteroylmonoglutamates and polyglutamates (ng/g)	Pteroylmonoglutamates (ng/g)	Squame weight per 24 h
T.R.I.	650	350	Not done
S.O.H.	550	130	Not done
M.O.T.	270	150	Not done
L.H.O.	250	150	Not done
B.E.R.	520	150	Not done
L.A.B.	450	150	Not done
K.A.R.	570	300	Not done
A.L.	575	130	Not done
A.L.B.	630	320	50g or 32 μg/24 h
Mean	495	200	

Folate content of squames

One measurement corresponding to the loss of squames in 24 h (50 g of squames) was made in a patient with generalized psoriasis, the other measurements were made on the folate content of 1 g of squame (Table 3).

DISCUSSION

The percentage of patients with low serum folate and low red cell folate has been found to be higher than reported by Fry *et al.* (1971), who observed a reduction of serum folate in 27% and of erythrocyte folate in only 4% of patients with psoriasis. Moreover, our figures are lower than those reported by Shuster, Marks & Chanarin (1967), who found low serum folate in 75% of their patients. As in Shuster's series, we were unable to find any correlation between the serum folate and the extent of the psoriasis.

Anaemia is rare in psoriasis and our results are in agreement with previous data. When the patient was anaemic, there was a good correlation with the reduction of serum folate. However, there was no correlation between the serum folate level and macrocytosis.

The mechanism of the folate deficiency found in all studies, although with variable frequency, has been discussed. Shuster & Marks (1970) considered that a dermatogenic enteropathy caused a malabsorption of folic acid by the small intestine. This opinion was supported by Summerly & Giles (1971) who demonstrated a malabsorption of folic acid in 43% of psoriatic patients. However, this incidence and the very existence of psoriatic enteropathy have been matters for debate. Preger *et al.* (1970), using several absorption tests and microscopical examination of jejunal mucosal biopsies, could not demonstrate a malabsorption syndrome in eight patients with psoriasis. The sample is probably too small to refute the existence of psoriatic enteropathy; however, it would seem that psoriatic enteropathy is too rare to be the explanation of folic acid deficiency in so large a proportion of cases of psoriasis.

Hild (1969) studied the loss of folic acid in six patients with psoriasis and mycosis fungoides who had low serum folate, and was unable to detect any malabsorption. Moreover, he found that the loss of folate in the desquamated squames was very high and, in his view, could account for the folate deficiency. Fry *et al.* (1971) repeated a similar study in fifty-eight cases of psoriasis and observed no evidence of malabsorption in twelve patients whose folate absorption was measured. The levels of folate found by these authors in the psoriatic squames (0·05–0·55 μg/g) are 10–80 times lower than the values reported by Hild.

Our study of folic acid absorption indicates there are two very different groups of patients.

In four patients, folate absorption results were grossly abnormal, with a flat uptake curve, indicating malabsorption in the small intestine. The folic acid absorption test is considered to be a reliable indication of the absorptive function of the small intestine. This type of flat curve is a constant feature of coeliac disease and lymphosarcoma of the small intestine, and is virtually constant in Whipple's disease (Zittoun *et al.*, 1973). These four results corroborate Shuster's hypothesis for the existence of a psoriatic enteropathy. Unfortunately, we have no other evidence (xylose absorption, X-ray examination, or biopsy) in these four patients to support the diagnosis of an enteropathy. It is of interest that three of these patients had severe psoriatic arthritis, and one was grossly obese.

All the other patients in whom a folic acid absorption test was carried out showed increased folate absorption. This enhanced response was probably due to increased absorption, although pharmacological and not physiological doses of folic acid are used in this test (Zittoun *et al.*, 1973). It is reasonable to suggest that the hyperabsorption compensates for an increased requirement.

The different theories are compatible with the discordant evidence of Hild on the one hand, and Fry and his colleagues on the other. Using Hild's techniques to measure squame folate, we observed values fairly close to those reported by Fry. We collected the squames shed during a 24 h period from only a single patient with generalised psoriasis, and obtained 50 g. This is clearly higher than normal. Croft, Lim & Taylor (1968) observed that the weight of squames desquamated in 24 hours was 14–28 g in extensive, and even generalized, psoriasis. Fry reported a similar figure. We consider, in view of our results and those of Fry, that the loss of folate in the squames is insufficient to be the only explanation of the serum folate deficiency.

We have not tested the folate content of normal skin: indeed the sampling of the keratinized layers of normal skin without contaminating the specimen with blood is almost impossible, and it would give very dubious results.

In conclusion, we have confirmed the observation of several earlier workers that a moderate folate deficiency occurs in psoriasis. Among the mechanisms proposed, folate loss in the squames is too low to be the sole explanation of this deficiency. Malabsorption and increased utilization can play a role either separately or simultaneously.

Four of twenty-six patients had malabsorption; found mainly in extensive psoriasis with crippling psoriatic arthropathy. All the others had increased folate absorption whatever their serum folate. Folate deficiency seems to be mainly due to increased utilization of folate for DNA synthesis by the epidermal cells in rapid turnover, as has been suggested by Fry *et al.* (1971). It is possible that the increased absorption is a compensatory mechanism which maintains the folate balance constant.

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Experimental studies on Methotrexate and rat liver

P.C.H.NEWBOLD

Department of Medicine, Postgraduate Medical School,
Hills Road, Cambridge CB2 2QL

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SUMMARY

Acute administration of MTX suppresses dihydrofolate reductase (DHFR) activity in skin and liver for 8 h, but there is later a reactive increase. Chronic administration is accompanied by increased DHFR, and little tissue storage of MTX—at least in liver and kidneys. Partial hepatectomy normally stimulates DNA synthesis as regrowth occurs. Simultaneous MTX administration lowers the DHFR response, but increases that of thymidine kinase and of thymidylate synthetase. There is also reduction of deoxyuridine incorporation into hepatic DNA. These results are discussed with reference to the chemistry and treatment of psoriasis.

Both the value of Methotrexate and its potential for harm are now well known. Although liver toxicity is important, very little basic research has been done, and the limited data available allow of conflicting interpretations. Convenience requires that rats should be used in these experiments, but it is essential to remember that these are fur-bearing animals which routinely indulge in coprophagy. This habit complicates all studies of folates and anti-folates, but suitable alternatives are not readily available.

The liver regenerating after partial hepatectomy is a convenient model for studying increased DNA synthesis. The effects can be enhanced by pre-treatment with growth hormone, or by preliminary surgery before the main operation (Moolten, Oakman & Bucher, 1970). Enzyme response begins about 12 h after operation, and Methotrexate has been reported to increase thymidylate synthetase, but to depress thymidine kinase, thymidylate kinase, DNA polymerase, and deoxycytidylate deaminase (Labow, Maley & Maley, 1969). Some authors have found that Methotrexate inhibits dihydrofolate reductase without inhibition of liver regrowth (Ngu, Roberts & Hall, 1964), while others have found little effect of Methotrexate, either on dihydrofolate reductase or on liver regrowth (Brown, Neal & Williams, 1965). Methotrexate injected 12 h after partial hepatectomy inhibits incorporation of orotic acid into DNA, so it does influence pyrimidine biosynthesis (Coward, Morse & Gentry, 1968). Surgical technique, and the amount of liver removed, will obviously lead to some variation, and different dose schedules, and order and timing of their administration can also explain some discrepancies. In humans there is an extremely wide variability of individual tolerance to Methotrexate, and toxicity increases with age (Hansen *et al.*, 1971). For rats also, age is important, and there is a significant effect from the normal circadian rhythm (Barbiroli & Potter, 1971). For the present experiments, animals of 3 months' age were used, and the operations were done in small groups at the same time each day. Feeding had to be *ad libitum* to allow for different recovery rates from the anaesthetic. Different cell lines vary in their reaction to Methotrexate, and enzyme data may be complicated by elution of Methotrexate during the isolation procedures, as it is not known where the Methotrexate is active (Roberts & Wodinsky, 1968). No significant difference was found between the sexes, nor was the channel of administration of obvious importance.

MATERIALS AND METHODS

Sprague–Dawley rats were used throughout, and these were reared from birth with unrestricted diet. Partial hepatectomy was by the method of Higgins & Anderson (1931), except that halothane was used in place of ether anaesthesia, as this reduces surgical mortality to around 5%. Approximately 70% of the liver was removed, but occasional anatomical anomalies required a reduction of this figure. Parenteral Methotrexate (Lederle Laboratories) was used without further purification. Deoxyuridine-6-^{3}H was from Amersham/Searle, and had a specific activity of 5 Ci/mmol. Thymidine-methyl-^{3}H was from ICN Radiochemicals, Irvine, California, and had specific activity of 8·2 Ci/mmol. Ribonuclease was obtained from Worthington, pyruvate kinase from Calbiechem, and all other chemicals were analytical grade.

Homogenization of tissues was in 50 mM tris-chloride buffer, pH 7·5, using a Virtis 45. The speed was slowly increased to full, maintained for 30 s, then the sample was allowed to cool before a second period of 30 s blending. All samples were kept on ice throughout, to minimize enzyme loss. Assays of dihydrofolate reductase and of Methotrexate were carried out as previously described (Newbold & Stoughton, 1972).

Thymidine kinase was assayed by a method derived from those of Bollum & Potter (1959), and Bresnick & Karjala (1969). 1 ml of buffer, pH 7·5, contained: tris-chloride 1mM, ATP 10 μM, magnesium chloride 10 μM, phospho-enol-pyruvate 10 μM, and 10 international units of pyruvate kinase. Labelled thymidine (0·175 mCi) was incubated with 1·0 ml of buffer, and 0·25 ml of tissue extract, for 30 min at 37°C. The reaction was then stopped by rapid cooling to 0°C, and all phosphates were absorbed on to discs of DEAE cellulose paper. These discs were then washed successively with 1 mM ammonium formate, distilled water, 80% ethanol and absolute ethanol. The discs were then dried, counted for tritium, and the results expressed as μM of ^{3}H-thymidine incorporated per gram wet weight of tissue. The scintillation mixture was 5 g of butyl-PBD in 100 ml of Bio-Solv BBS 2 and toluene 900 ml. Counting efficiency was 35%.

Protein was measured by the biuret method, and DNA by Burton's method (1956). All results are expressed per gram wet weight of tissue, as the variability was so great when DNA or protein were used, that the effects of the Methotrexate were partially obscured. One variable causing this result is the extent of liver damage from anaesthetic, operation, thrombosis, infection and drug. All animals who failed to eat and looked ill were excluded from the series. Thymidylate synthetase was assayed by the method of Wahba & Friedkin (1961), as modified by Dunlap, Harding & Huennekens (1971). The methylene tetrahydrofolate was stabilized with mercaptoethanol, and the increase in optical density at 340 nm was measured at 37°C in a Pye-Unicam spectrophotometer, series SP 1800. Under the conditions of the assay, one unit of enzyme will synthesize 1 μmol/min of thymidylate.

For the study of deoxyuridine incorporation, the organs were frozen quickly, homogenized, and the DNA extracted by the Marmur (1961) technique, as modified by White (1971). The yield was about 90 mg DNA per 50 g of rat liver, which accords with Kirby's findings (1957). Counting efficiency, with internal standardization, was 35%.

RESULTS

Acute effects of Methotrexate

To provide a baseline, 3-week-old rats of weight 95–100 g were each injected intra-peritoneally with 575

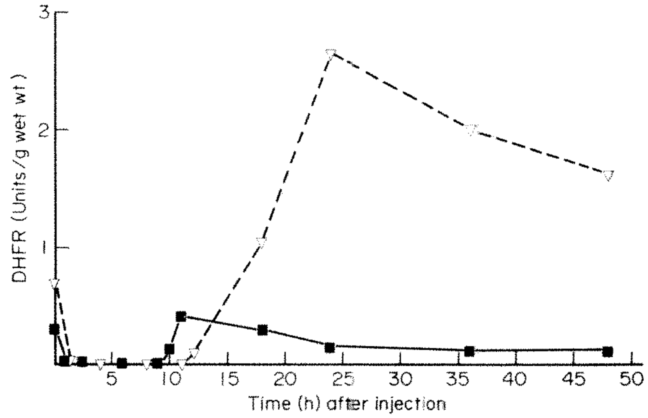

FIGURE 1. Acute effect of Methotrexate upon skin and liver dihydrofolate reductase. Each 3-week-old rat was injected with 575 μg MTX at time zero. ▽ – – – – ▽, skin; ■———■, liver.

μg Methotrexate, then sacrificed at intervals. The livers and skin were homogenized separately, then assayed for dihydrofolate reductase, thymidylate synthetase, protein and DNA. Fig. 1 summarizes the data, and each point is derived from the average results from at least five animals. Thymidylate synthetase was not assayable except at time 8 h, so it is not included in the figure. In a young rat whose fur is erupting, the skin is an actively metabolizing organ; this explains part of the excess uptake of Methotrexate when compared with the liver. Even so, it is possible that proteases reduced the figure more for the liver than for the skin, despite strict control of temperature.

Chronic effects of Methotrexate

Table 1 summarizes the data from twenty rats treated over a period of 22 months, and ten controls. Each group consisted of five male and five female rats. The Methotrexate-treated groups were either given 0·1 mg/kg or 0·4 mg/kg, on a schedule of 5 days on the drug and 9 days off. These animals all had intraperitoneal colchicine, 2 mg/kg (which was given because other workers were studying mitoses and chromosome structure), $4\frac{1}{2}$–5 h before sacrifice by ether anaesthesia and exsanguination. This is known not to influence the assays in this group, and no other animals were given colchicine. In the high treatment group, one kidney result was excluded, as it gave the unexplained dihydrofolate

TABLE 1. Effect of chronic feeding of MTX to rats. Details in text

	Tissue	DHFR (Units/g)	MTX/g wet weight
Controls	Liver	0·92 (0·08)*	—
	Kidney	2·33 (0·36)	—
	Intestine	0·72 (0·04)	—
MTX	Liver	5·76 (1·37)	$38{\cdot}38 \times 10^{-7}$ M (14·5)
0·1 mg/kg body	Kidney	2·31 (0·63)	$5{\cdot}10 \times 10^{-7}$ M (1·29)
weight	Intestine	0·81 (0·16)	$2{\cdot}30 \times 10^{-8}$ M (0·71)
MTX	Liver	1·92 (0·30)	$16{\cdot}23 \times 10^{-7}$ M (7·26)
0·4 mg/kg body	Kidney	2·51 (1·10)	$3{\cdot}05 \times 10^{-7}$ M (1·82)
weight	Intestine	1·12 (0·27)	$2{\cdot}80 \times 10^{-8}$ M (1·23)

* Standard deviations are given in brackets.

reductase value of 46 units/g. Both liver and kidney seem to adapt to the presence of Methotrexate by increasing dihydrofolate reductase, but this is not obviously related to the amount of Methotrexate administered, nor the amount stored in these organs. The intestine has not been shown to share in this phenomenon, but perhaps the normally high turnover of cells, and the presence of proteases, may invalidate some of the findings.

Partial hepatectomy experiments

These were all performed in 3-month-old rats, the sexes being kept separate from infancy to remove the risk of pregnancy. Control animals were anaesthetized, the liver manipulated, and the abdomen was then closed. For the dihydrofolate reductase study, animals were sacrificed at the times indicated in Fig. 2, while the treated animals were injected intra-peritoneally with 1·0 mg of Methotrexate at the end of the operation. This is approximately 4 mg/kg body weight. Repeated doses, of different schedules, gave increased mortality with such poor survival that results were impossible to analyse. So all injections were carried out at time zero. Fig. 3 shows the results for thymidine kinase, and Fig. 4 for thymidylate synthetase, both with and without Methotrexate. For the deoxyuridine experiments, partial hepatectomy was carried out as above. Half the animals had Methotrexate 5 mg intra-peritoneally at time 24 h, then 200 μCi of ^{3}H-deoxyuridine at 25 h, and were sacrificed at 26 h. The other half had the deoxyuridine alone at 25 h, that is 1 h before sacrifice. DNA was extracted from livers and kidneys as described, and counted for tritium. The results are expressed in Table 2.

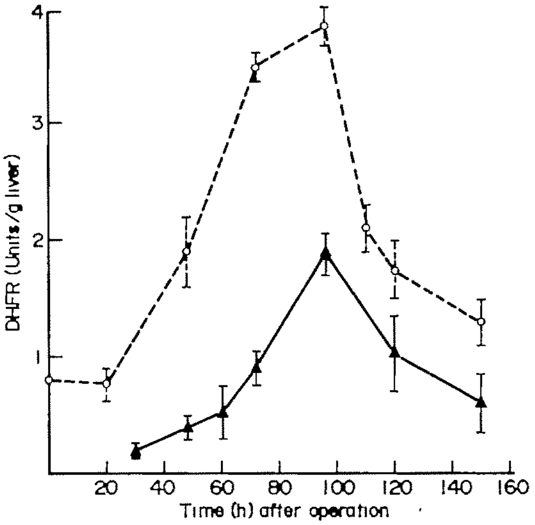

FIGURE 2. All rats were subjected to partial hepatectomy. The MTX group had 1 mg MTX intra-peritoneally at time zero. Liver dihydrofolate reductase values are expressed in units per gram wet weight. Each point refers to 8–10 animals, and standard deviations are shown. ○ - - - - ○, without MTX; ▲——▲, with MTX.

DISCUSSION

The increase in activity of both thymidine kinase and thymidylate synthetase, with suppression of dihydrofolate reductase, indicate that the inhibitory effect of Methotrexate is total for about 8 h. The results also show that the compensatory mechanisms are active for at least 100 h thereafter, so

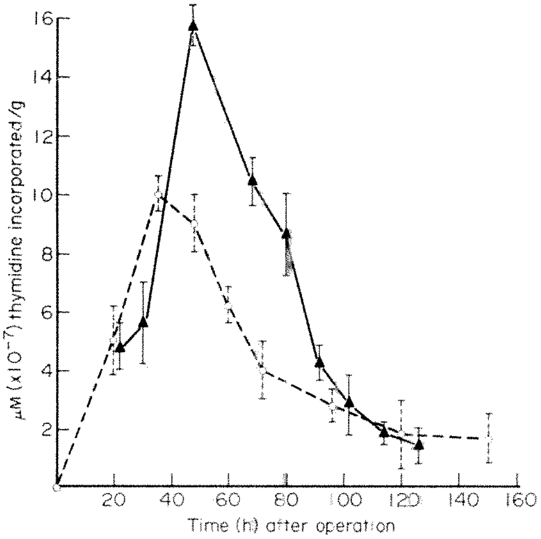

FIGURE 3. Thymidine kinase levels after partial hepatectomy, both with and without MTX, 1 mg intra-peritoneally, at time zero. Each point refers to 6–8 animals, and standard deviations are shown. ○ – – – – ○, without MTX; ▲———▲, with MTX.

TABLE 2. Incorporation of deoxyuridine into DNA of rats after partial hepatectomy. All animals had 200 μCi ^{3}H-deoxyuridine 1 h before sacrifice, and the MTX group had 5 mg intra-peritoneally 24 h after operation

Counts/min per mg DNA	SD	*P* value
Livers		
No MTX 2051	± 815	
+ MTX 1368	± 341	0·01
Kidneys		
No MTX 5451	±2415	
+ MTX 2479	± 980	0·05

experiments with repeated injections inside the period of action of the first dose of Methotrexate will show additive effects and toxicity. The regenerating rat liver is an imperfect model for human psoriasis, as the liver is the major store of folate in the body, and as such is the normal centre of activity for folate-dependent enzymes. No matter how fast the cell turnover, the skin never can act as a major store of folate. Nevertheless, such studies can help to evaluate different treatment regimes. It is apparent that further studies are needed to evaluate the effects of stress, intercurrent illness and treatments, besides the circadian rhythms of the patient and his disease. A further point is that Methotrexate considerably increased post-operative mortality by a mechanism which cannot be explained. Although Methotrexate is rapidly and nearly totally eliminated, it can produce a chemical lesion that long outlives its presence.

Fig. 1 showed the rapid disappearance of DHFR, which is one of its first effects, and that the liver recovers the ability to synthesize enzyme more rapidly than the skin. Both show a rebound phenomenon, which seems to be more prolonged in the skin than in the liver. The stress of liver regrowth is so great that the pool of available enzyme reduces the inhibitory effect of the antifolate, although the normal peak is widened. Fig. 3 shows that thymidine kinase is first depressed, then stimulated, to exceed the control levels after 8 h. Fig. 4 indicates that thymidylate synthetase is increased, and falls only when the level of dihydrofolate reductase is recovering. Hence, one explanation of the differing individual tolerance of Methotrexate is in the ability to produce these compensatory enzymes while the target is reeling. The ability to respond by increased production of thymidine kinase and thymidylate synthetase may be genetically determined, as may the ability to co-ordinate these enzymic mechanisms. More knowledge is needed in the fields of chemical controls, enzyme regulation, and the role of inheritance.

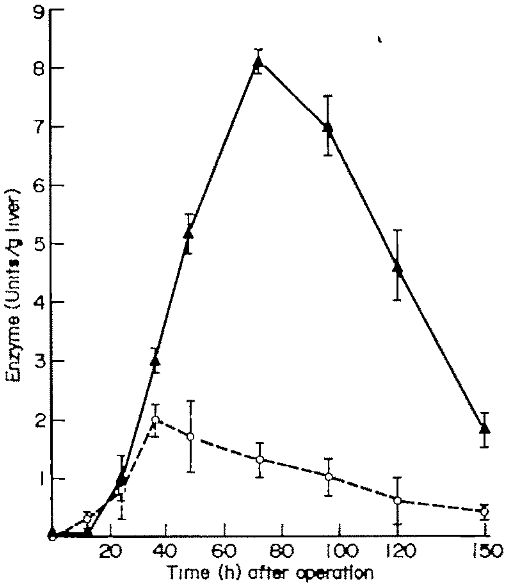

FIGURE 4. Thymidylate synthetase levels in rat liver after partial hepatectomy. Controls had no MTX. In the treated group all had 1 mg intra-peritoneally at time zero. Each point refers to 6–8 rats, and standard deviations are shown. ○ – – – – ○, without MTX; ▲———▲, with MTX.

When this basic knowledge is accumulated, it will help in the management of psoriasis and other diseases with increased epidermal activity. Any differences between the skin and other organs in handling DNA synthesis and its precursors, both in normals and in psoriatics, might be exploitable to the patient's advantage. But systemic treatment with such powerful agents cannot be recommended until some means of screening other susceptible tissues of the body has been developed.

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Photosensitivity due to the 'sunburn' ultraviolet content of white fluorescent lamps

A. KOBZA, C. A. RAMSAY AND I. A. MAGNUS

Institute of Dermatology, Homerton Grove, London E9 6BX

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SUMMARY

Six photosensitive patients are described, who also showed skin sensitivity to white fluorescent lamps. The results of tests with monochromatic irradiation showed that all patients were very sensitive to the 'sunburn' part of the spectrum (300–320 nm) with a lowered minimal erythema dose and a delayed papular-type reaction.

Tests with a number of cut-off glass filters demonstrated that the fluorescent lamp sensitivity in these patients was due to the small amount of 'sunburn' UVR emitted. Spectral power analysis of the lamps showed that, with the exposures used in our tests, sufficient 300–320 nm UVR is emitted to produce skin reactions in very sensitive persons.

There are few reports in the literature of skin photosensitivity from white fluorescent lamps. In those which have been published, causative wavelengths have been in the visible spectrum, causing solar urticaria (Lamb *et al.*, 1958); or the sensitivity has been to long wave ultraviolet radiation (UVR), producing solar urticaria and delayed reactions (Brown, Lane & Magnus, 1969). However, both Brown *et al.* (1969) and Bresler (1949) suggested that wavelengths shorter than 320 nm emitted from fluorescent lamps could cause cutaneous reactions.

We have recently investigated six patients with various photodermatoses, who were very sensitive to UVR around 290–320 nm, i.e. wavelengths which give the normal sunburn response. These patients reacted to white fluorescent lamps. Spectral power distribution analysis of these lamps showed that the small amount of 'sunburn' UVR emitted was responsible for the reaction.

METHODS

Action spectra were determined by irradiating the skin with narrow waveband radiation from a prism monochromator using a 2 kW xenon arc source (Magnus *et al.*, 1959).

Skin tests with fluorescent lamps. Areas of skin 3 × 3 cm were irradiated for 30 min with a bank of four 2 ft 20 W white fluorescent lamps placed 30 cm from the skin. Sites were covered with 2 mm thick Schott glass filters, WG 360, WG 345, WG 335, WG 320, and WG 305, equivalent to the older WG2, WG3, WG4, WG5, and WG6 respectively. Filter WG1 was also used, for which there appears

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Correspondence to Dr Ramsay.

to be no present equivalent in the recent Schott filter catalogue. Unfiltered skin areas were irradiated at the same time for 30 min.

Absorption spectra of glass filters were measured in a Unicam SP.800 recording spectrophotometer.

Spectral emission curves of the fluorescent lamps were measured with a Beckman DB-G recording spectrophotometer. The sensitivity and efficiency of the photomultiplier and optical system of this instrument vary with wavelength, and the vertical axes of the recordings are uncorrected.

Spectral power distribution measurements on the white fluorescent lamps used in our tests, and on four 2 ft 20 W 'black light' lamps, were carried out in the laboratories of a large lighting manufacturer. The instrument used for these measurements was a modified spectrophotometer, calibrated using a ribbon filament tungsten lamp with a quartz window, operated at a colour temperature of 2600°K. The calibration factor at each wavelength was then applied to the reading of the lamp under test.

CASE REPORTS AND RESULTS OF ACTION SPECTRA STUDIES ON PATIENTS

The minimal erythema dose (MED) in normal adults at 300 nm was found by Magnus (1964) to range from about 9 to 120 mJ/cm^2.

Case 1

T.H. (P.6404), female, aged 57. Born in India of Indian parents, she showed a moderate degree of racial pigmentation. She had been resident in this country since 1948. Photodermatitis commenced in 1971, as an eczematous eruption confirmed by histology, and has persisted to date. Several exacerbations have occurred, and oral prednisone was commenced in September 1971; the dose at time of our tests was 5 mg daily. A number of drugs had been taken before and after the onset of her eruption (imipramine, chlordiazepoxide, pseudoephedrine, tetracycline, erythromycin, and 'Nargesic'), but the photosensitivity persisted when the drugs were stopped. Photopatch tests performed elsewhere were difficult to interpret, because the normal skin reacted to radiation from the 'black light' lamps used for these tests. It was, therefore, suspected that she was sensitive to long wave UVR, as 'black light' lamps have a high output in this part of the spectrum.

Monochromator tests showed abnormal results limited to wavelengths between 300 and 320 nm. The abnormality consisted of a lowered MED, together with a papular type response to a low dose at these wavelengths. Tests at all other wavelengths (340–700 nm) were negative.

Case 2

G.H. (G.1809), male, aged 60. A photosensitive eruption, suggestive of 'actinic reticuloid', started in 1967 with extensive erythema, scaling and lichenification of exposed sites. Prednisone, 40 mg daily, was commenced in 1967 and slowly reduced to the present dose of 15–20 mg daily. Histology of skin from the face showed a dense nodular infiltrate of lymphoreticular cells in the dermis. The epidermis was moderately thickened, but did not show obvious eczematous change.

Monochromator tests showed lowered MED at 300 and 307·5 nm, with abnormal papular responses at all wavelengths between 300 and 500 nm. Sensitivity in the 300–320 nm range was particularly marked.

Case 3
C.T. (M.54969), male, aged 64. 'Actinic reticuloid' type of photosensitivity developed in 1967, with marked lichenification and erythema of exposed areas. The initial prednisone dose of 40 mg daily, was later reduced to 7·5 mg on alternate days.

Past history. Recurrent dermatitis of the hands since 1940.

Monochromator tests. Lowered MED at 300–320 nm and abnormal morphological reaction at all wavelengths between 300 and 600 nm. Marked sensitivity at 300–320 nm.

Case 4
J.B. (P.7656), male, aged 55. Photosensitivity, 'actinic reticuloid' in type, commenced in 1970. Histology of skin from the forehead showed focal spongiosis and parakeratosis with an irregular acanthosis. There was oedema of the papillary dermis with a moderately dense infiltrate around blood vessels. The infiltrate was mixed, but contained a few larger mononuclear cells.

Past history. Jessner's lymphocytic infiltration of the skin in 1958, which cleared.

Monochromator tests. Lowered MED at 300–320 nm with abnormal papular type response at all wavelengths between 300 and 500 nm. Sensitivity particularly marked at 300–320 nm.

Case 5
G.C. (P.4514), male, aged 46. Photosensitivity of 'actinic reticuloid' type started in 1965. There were patchy erythema and some lichenified papules on exposed areas. Histology of skin from the back of the neck showed lichenification, considerable solar elastosis, and a moderately dense round cell infiltrate in the upper dermis.

Past history. Recurrent eczema of palms and soles for 20 years.

Monochromator tests. Abnormal papular responses at all wavelengths tested between 300 and 600 nm. Sensitivity marked at 300 and 307·5 nm, where the MED was lowered.

Case 6
R.S. (M.27464), male, aged 42. Photocontact dermatitis to tetrachlorosalicylanilide (TCSA) in 1960 with positive photopatch test. The skin cleared completely when contact with the chemical was stopped. In 1965 an 'actinic reticuloid' type of photosensitivity commenced and has persisted. Prednisone 20 mg daily was started in 1968, the present dose being 5 mg on alternate days. Patch tests in December 1971 were positive to TCSA and *p*-aminobenzoic acid (PABA). Photopatch tests were positive to TCSA, trichlorocarbanilide, PABA, bithionol, hexachlorophane and fenticlor. The reactions to TCSA and PABA with 'black light' radiation were more marked than the non-irradiated test sites with these chemicals.

Monochromator tests. Marked sensitivity at 300–320 nm with lowered MED. Abnormal morphological responses of the papular type at 300–700 nm.

Case 7
E.B.-L. (E.9120), male, aged 62. Contact dermatitis on hands and feet in 1961, due to rubber; it

settled with treatment and avoidance of rubber. In 1971 dermatitis started on the hands, spreading to most of the body, but more marked on light exposed areas. Histology of skin from the dorsum of the hand suggested a chronic lichenified eczema.

Monochromator tests. Sensitivity limited to 300–320 nm; the MED was within normal limits, but abnormal papular type reactions developed at a dose of three times MED. Tests at all other wavelengths were negative.

Case 8

T.H. (P.7652), male, aged 68. For 40 years he had suffered from a rash on the face in the summer months after exposure to sunlight. It was clear in winter. Since 1971 he had had a more marked eruption on light exposed areas which started in the winter and which has persisted. Histology of skin from the back of the neck and the dorsum of a hand showed lichenified eczema.

Monochromator tests. Sensitivity limited to 300–320 nm. The threshold dose at these wavelengths was within normal limits, but an abnormal papular type reaction developed at the threshold dose. Tests at all other wavelengths were negative.

TABLE 1. Threshold dose at various wavelengths in 'sunburn' UV spectrum as determined with the monochromator

	Threshold dose (mJ/cm²)		
Case no.	300 nm	307·5 nm	320 nm
1	2 4	12·5	62·3
2	<1·6	<3·8	228
3	1·7	2·5	12·5
4	3·5	5·2	13·5
5	1·4	9·3	177
6	1·7	2·4	4·5
7	11·8	208	312
8	52	75	135

The threshold dose in each patient, as determined with the monochromator at 300, 307·5 and 320 nm, is given in Table 1. Only one patient (Case 1) had noted exacerbation of his dermatosis from fluorescent lamps at work.

OTHER RESULTS

Transmission spectra of glass filters

These are shown in Fig. 1.

Filters WG1, WG 360 and WG 345 have 50% transmission at 335 nm and above. WG1 has virtually zero transmission at 355 nm, WG 360 at 335 nm, and WG 345 at 320 nm; these filters thus absorb virtually all of the 'sunburn' UVR. Conversely, filters WG 335, WG 320 and WG 305 all transmit varying amounts of 'sunburn' UVR. At 313 nm WG 335 transmits approximately 15%,

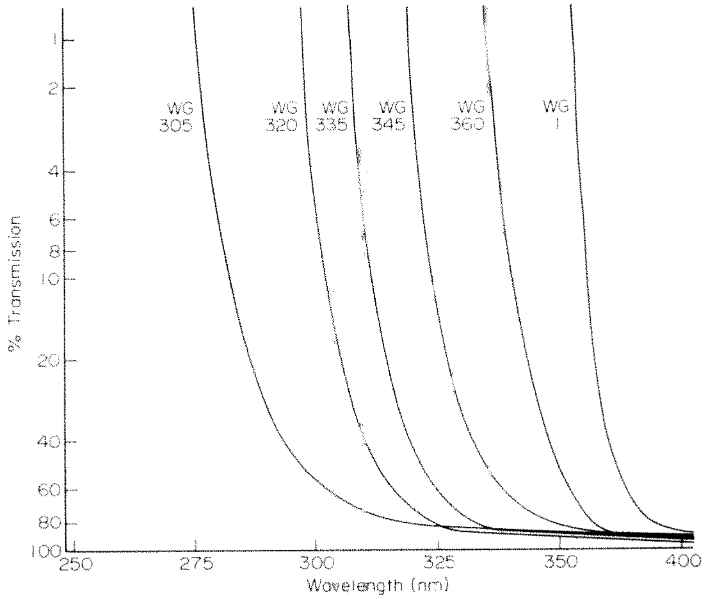

FIGURE 1. Transmission spectra of glass filters.

WG 320 55%, and WG 305 75%. At 302 nm the transmission of WG 335 is virtually nil, WG 320 transmits approximately 10%, and WG 305 60%.

Irradiation of the skin with white fluorescent lamps

Tests with these lamps without filters gave a delayed erythematous or papular type of reaction which appeared several hours after an exposure of 30 min in six patients (Cases 1–6). There were no immediate erythematous or urticarial reactions. Tests with and without filters were negative in two patients (Cases 7 and 8). The patients who reacted to unfiltered radiation all showed similar responses to tests using one or more glass absorption filters (Table 2). Tests with filters WG1, WG 360 and WG 345 were consistently negative, whereas tests with those filters which transmitted some 'sunburn'

TABLE 2. Results of tests with white fluorescent lamps

Case no.	Unfiltered radiation	Filters giving erythematous or papular reactions*
1	+ve	WG 305
2	+ve	WG 305 and 320
3	+ve	WG 305, 320, and 335
4	+ve	WG 305
5	+ve	WG 305, 320, and 335
6	+ve	WG 305 and 320
7	−ve	All −ve
8	−ve	All −ve

* All filters giving positive reactions have 50% transmission between 297 and 322 nm.

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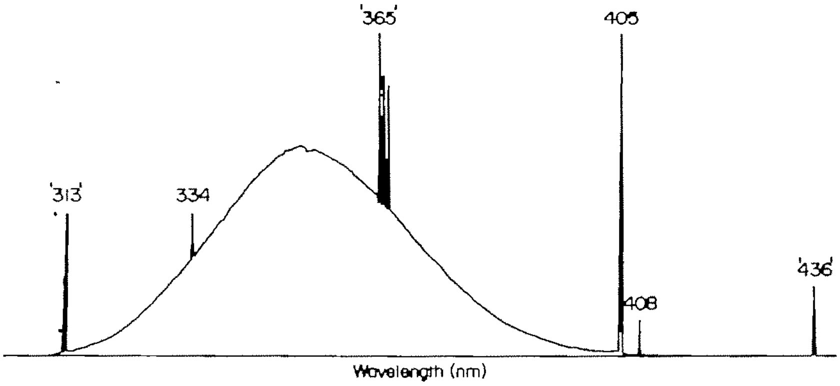

FIGURE 2. Spectral emission curve of 2 ft 20 W 'black light' fluorescent lamp, from 300 to 440 nm. Slow scan, slits 0·05 mm.

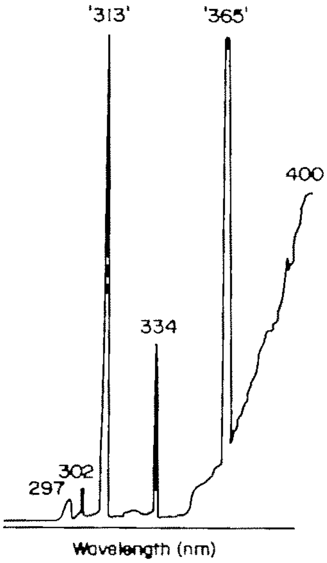

FIGURE 3. Spectral emission curve of 2 ft 20 W white fluorescent lamp, from 295 to 400 nm. Fast scan, slits 0·2 mm.

TABLE 3. Calculated power output (mW/cm^2) at various wavelengths with white and 'black light' fluorescent lamps. Values are the mean of four lamps

Wavelength (nm)	White lamps	'Black light' lamps
300	$2{\cdot}3 \times 10^{-6}$	$2{\cdot}0 \times 10^{-4}$
302·2	$6{\cdot}8 \times 10^{-4}$	$1{\cdot}1 \times 10^{-4}$
313·2	$7{\cdot}2 \times 10^{-3}$	$2{\cdot}0 \times 10^{-3}$
320	$6{\cdot}1 \times 10^{-5}$	$3{\cdot}2 \times 10^{-2}$

UVR (WG 335, WG 320 and WG 305) were positive. Positive tests developed in all patients with WG 305, in four patients (Cases 2, 3, 5, and 6) with WG 320, and in two (Cases 3 and 5) with WG 335 filters. Thus, the erythematous or papular reactions produced by the unfiltered lamps were due to their 'sunburn' UVR output.

Spectral emission curves of the fluorescent lamps are shown in Figs. 2 and 3.

Spectral power distribution analysis of fluorescent lamps

White lamps. These measurements showed that some power, albeit extremely small, was present from wavelengths below 300 nm, in fact down to 270 nm, the shortest wavelength tested. In the terrestrial sunburn UVR region (300–320 nm) the power was variable, depending on the wavelength (Table 3). Most power in this region was present at 302·3 and 313·2 nm, the position of the mercury 'lines' in this region. The approximate dose received by our patients at various wavelengths from the four fluorescent lamps with an exposure time of 30 min is shown in Table 4.

TABLE 4. Calculated dose given (mJ/cm²) to the skin with white and 'black light' fluorescent lamps. Values are the mean of four lamps, 30 cm from the skin, with an exposure time of 30 min

Wavelength (nm)	White lamps	'Black light' lamps
300	0·04	0·37
302·2	1·2	0·2
313·2	13·0	3·6
320	0·12	58·0

'Black light' lamps. Although these lamps were not used for testing our patients in this particular study, we give the power distribution measurements, which were rather different from the white lamps. As shown in Table 3, the power present at 300 nm was greater, but at 302·2 and 313·2 nm it was much less than with the white lamps. However, at 320 nm, the approximate upper limit of 'sunburn' UVR, the power was much greater. The approximate dose at these wavelengths that would be received from four of these lamps with a 30 min exposure is shown in Table 4.

DISCUSSION

The first report of photosensitivity from fluorescent light was by James (1941), who described two patients who developed a dermatitis whilst working 3–8 feet beneath fluorescent lamps. The evidence incriminating these lamps was strong, but circumstantial. No skin tests were performed with the lamps and no information was given to enable the responsible part of the spectrum to be delineated.

Bresler (1949) gave details of five female patients who developed itching, erythema, and dryness of the skin, whilst working in very close proximity to 'daylight' fluorescent lamps for several hours a day. The condition cleared completely when the lamps were fitted with a plate glass shield, suggesting that these patients had suffered from normal 'sunburn'.

The cases of Lamb *et al.* (1958) were of dermatoses induced by visible light, there being no evidence of sensitivity to 'sunburn' UVR.

A much more detailed study of photosensitivity from fluorescent lights was made by Brown *et al.* (1969), who described five patients. All were sensitive to long wave UVR, as determined by monochromator tests, and it was thought that this part of the spectrum was responsible for the fluorescent light reactions. In addition, however, it was strongly suspected that three patients, one with actinic reticuloid and two with solar urticaria, also reacted to the 313 nm 'line' from the lamps.

In the present study, it was first suspected that 'sunburn' UVR might be responsible for the positive fluorescent light tests after narrow wave band irradiation with the monochromator had been carried out in the first patient (Case 1). This woman had reacted adversely to the 'black light' irradiation source alone, when it was used for photopatch tests, and it was, therefore, suspected that she was sensitive to long wave UVR, because of the spectral emission curve of these lamps (Fig. 2). However, monochromator tests showed no reaction above 320 nm, although she was very sensitive to wavelengths below 320 nm. Tests with white fluorescent lamps were positive, and that this reaction was due to wavelengths below 320 nm was confirmed by the tests with absorption filters. The spectral emission curve of a white fluorescent lamp is shown in Fig. 3.

This result led us to carry out tests with white fluorescent lamps, with and without filters, in a number of other patients with sensitivity to 'sunburn' UVR. It soon became obvious that only patients with a low threshold dose in this part of the spectrum gave positive results. When sensitivity was manifest by a papular reaction, but with a threshold dose that was within normal limits for erythema, white fluorescent lamp tests were negative. The dose received from these lamps in the positive tests, as calculated from the spectral distribution figures, correlated well with the monochromator results (Tables 1 and 4). It should be noted from Table 1 that the actual threshold dose at 302·3 nm was not estimated with the monochromator. The half band pass of our instrument is approximately 3 nm at 300 nm where tests were performed, and 302·2 nm would therefore be included within this band pass. We were not able to measure spectral power distribution of the actual 'black light' lamps used for photopatch tests in Case 1, but results with similar lamps from the same manufacturer are shown in Table 3. It is very probable that the initial positive reaction to the 'black light' lamps in Case 1 was due to radiation at the 320 nm region. The approximate dose that she would have received at this wavelength (Table 4) correlated with her threshold dose, as determined with monochromatic radiation (Table 1).

Fluorescent lamps consist of a low pressure mercury arc enclosed in a glass tube, the wall of which is very thin (approx. 1 mm thick). The inside of the tube is coated with phosphors which absorb the 254 nm radiation of the mercury arc and fluoresce, emitting radiation of longer wavelengths. Although the great majority of the output of a low pressure mercury arc is at 254 nm, other 'lines' are present, the important ones in the terrestrial sunburn UVR region being at 302·2 and 313·2 nm.

It should be stressed that values given for spectral power and dose are approximate; nevertheless the spectral power analysis of the fluorescent tubes used in the tests was the key to explaining our results. As shown in Table 4, a 30 min exposure with four white lamps gave a dose at 302.2 nm of approximately 1·2 mJ/cm^2, and four of our patients (Cases 2, 3, 5 and 6) had a threshold dose of this order at this part of the spectrum. It would thus seem almost certain that these patients reacted to the 302·2 nm line. The threshold dose at 300 nm in Cases 1 and 4 was somewhat higher than the calculated dose in this region received from the fluorescent lamps, and it is possible that the positive reaction with filter WG 305 in these patients was due to other parts of the 'sunburn' spectrum, perhaps the 313·2 nm 'line' which is also transmitted by this filter.

The reactions with filter WG 320 seen in some patients (Cases 2, 3, 5 and 6) could possibly be due in part to the 302·2 nm 'line', as this filter transmits a small amount (approx. 8%) of this line. A much more likely explanation, however, for the reactions with WG 320 and with WG 335 (seen in Cases 3 and 5) is that they were due to the 313·2 nm 'line'. WG 320 transmits 55% and WG 335 15% at this wavelength. We have no direct proof that the 313·2 nm 'line' was responsible in these patients, because we did not estimate the threshold dose with the monochromator at this wavelength, but it would explain our results.

What is definite, however, is that 'sunburn' UVR was responsible for all of the fluorescent light reactions seen in our patients. All of the filters used transmitted longwave UVR and visible light, but positive results were obtained only with those filters which also transmitted 'sunburn' UVR.

It is difficult to know the importance of the 'sunburn' UVR emission of white fluorescent lamps in perpetuating the photodermatitis in our patients. Knowledge that such wavelengths are emitted from these lamps, however, enables the best advice about domestic lighting to be given to photosensitive patients.

White fluorescent lamps from all manufacturers are similar in structure, and it is very likely that they will have a similar emission in the 'sunburn' UVR. The fitting of an opaque plastic shield around the lamps should provide complete protection against these wavelengths.

It should be emphasized that the number of persons in the general population who show sensitivity to white fluorescent lamps is probably extremely small. Terrestrial sunlight usually contains considerably more 'sunburn' UVR than the equivalent amount of total radiation from a white fluorescent tube.

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We should like to thank our colleagues who originally referred the cases.

Fig. 2 is reproduced from *British Journal of Dermatology* (1969) **81**, 420, by kind permission of the Editor.

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British Journal of Dermatology (1973) **89**, 361.

Effects of long wave ultraviolet radiation on photosensitizing and related compounds. I

THE EFFECTS ON THE FLUORESCENCE OF TOPICALLY APPLIED COMPOUNDS

MILHIM D.ALANI*

The Photobiology Program, The Skin and Cancer Hospital, Department of Dermatology,
Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140, U.S.A.

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SUMMARY

The effects of long ultraviolet radiation (UVA) on the fluorescence of twenty-four photosensitizing and related compounds were studied. Each compound was applied in two areas on the skin of hairless mice. One area was irradiated. The fluorescence in both areas was then inspected every day until it disappeared.

Persistence and change of colour of the fluorescence as a direct immediate effect of UVA radiation on compounds having photoallergic potential, i.e. salicylanilide, halogenated salicylanilide and phenothiazine derivatives, was observed. Compounds known for their phototoxic effects, i.e. acridine dyes, did not show such changes after irradiation, while 8-methoxypsoralen showed persistence of fluorescence without change of colour.

The fluorescence of most photosensitizing compounds has been known for many years, but very little is known concerning the effects of ultraviolet radiation on the fluorescence of these compounds, especially when applied on the skin. Therefore, it was considered of interest to compare the fluorescence of such compounds applied on the skin of hairless mice, before and after long wave ultraviolet (UVA) irradiation. Twenty-four fluorescent compounds were included in this study, nineteen of which were either potential photoallergens or structurally related thereto, the remaining five being phototoxic compounds (Hjorth & Fregert, 1968; Pathak, 1969).

MATERIALS AND METHODS

Test compounds

A 1% solution (10 mg/ml) of each compound in dimethyl sulphoxide (DMSO) was made. The compounds included salicylanilide, twelve halogenated salicylanilides (HS), six phenothiazine derivatives, four acridine dyes and 8-methoxypsoralen. These were obtained in reagent form from various suppliers (Table 1).

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* Visiting Research Instructor (Dermatology), Temple University, and PostdoctoralFellow, Åge Bang Foundation, Denmark.

Reprint requests to Dr Alani, Axgatan 6, 431 40 Mölndal, Sweden.

Experimental animals

140 hairless albino female mice, 10–12-weeks-old, derived from the CRH strain (Mann, 1971) were used. Each compound was tested on five mice.

Ultraviolet radiation sources

Irradiation lamp. Long wave ultraviolet radiation (320–400 nm) (UVA) was provided by a xenon arc solar simulator utilizing a Schott filter No. 3452 (formerly No. WG3, 2 mm thick), which filters out wavelengths less than 320 nm. The total incident ultraviolet dose, delivered during a 5 min exposure to a circular spot of 11 mm in diameter, was approximately 13 $J.cm^{-2}$ (Berger, 1969).

Examining lamp. A medium pressure mercury lamp with a low intensity, emitting principally in the spectral region of 253 and 366 nm ('Mineralight' No. UVS L13, Ultraviolet Products, San Gabriel, California), was used to detect fluorescence of the skin. The lamp was constructed so that one or both mentioned spectra could be used for inspection.

Experimental methods

A sample of 20 μl of test solution was pipetted onto 2 cm^2 of skin, in two areas, on the anaesthetized mouse, one sample on each side of the dorsal midline. 30 min later the right side was partially covered with opaque paper, leaving a spot 11 mm in diameter uncovered, which was then irradiated with the xenon arc solar simulator as described above. The skin of the animal was examined immediately after the irradiation as well as daily, under 'Mineralight', to detect any of the following; (a) alteration in intensity of the fluorescence, (b) change of colour, and (c) persistence of fluorescence. Further, the skin was inspected in daylight for any visible reaction.

Using the same above-mentioned experimental method, tetrachloro-salicylanilide (TCSA) was used in the following control experiments:

(1) The skin of a group of animals was irradiated before the application of TCSA.

(2) A second group was tested with *in vitro* irradiated TCSA.

(3) Both the skin of a third group and TCSA were irradiated separately and simultaneously before the application.

Further, as control experiment, the DMSO was tested alone on another group of animals.

RESULTS

The results of the study are summarized in Table 1.

Each tested compound when applied on the skin had a characteristic colour of fluorescence. Different compounds had different fluorescence persistence times, ranging from 1 to 5 days. After UVA irradiation the following changes in the exposed area were observed:

(1) Persistence and change in colour of the fluorescence seen with salicylanilide, all HS compounds and phenothiazine derivatives.

(2) No persistence or change in colour of the fluorescence was observed with all acridine dyes.

(3) Slight decrease in intensity of the fluorescence was observed with acridine orange and fluorescein compared with the control side. This decrease was undetectable after 1–2 days, so that the intensity of fluorescence in both irradiated and non-irradiated areas was the same thereafter, and lasted for the the same period.

(4) Persistence of fluorescence without change in colour was observed with 8-methoxypsoralen.

In the control experiment with TCSA, no persistence of fluorescence or change of its colour resulted when TCSA and/or the skin were irradiated separately prior to the application.

The skin of the control group tested with DMSO showed no flourescence before and after irradiation.

TABLE 1. The colour and persistence of fluorescence of twenty-four photosensitizing compounds applied on the skin of hairless mice before and after long wave ultraviolet irradiation

Tested compound	Initial fluorescence before irradiation.		Fluorescence after irradiation.	
	Colour	persistence (days)	Colour	persistence (days)
Salicylanilide and halogenated salicylanilides[a]				
Salicylanilide	Blue	1	Pale yellow	3
3,3′,4′, 5-tetrachloro- (TCSA)	Blue	1–2	Yellow	4–6
3,3′,-dichloro-				
3′,4′,-dichloro-				
3,5 -dichloro-				
4′,5 -dichloro-				
3 -chloro-				
3′-chloro-				
5 -chloro-				
3,4′,5-tribromo-(TBS)	Greenish-blue	1–2	Yellow	4–5
4′,5 -dibromo-				
4′ -bromo-				
5 -bromo-				
Phenothiazines				
Chlorpromazine hydrochloride (Thorazine)[b]	Blue	1	Orange-yellow	3
Prochlorperazine edisylate (Compazine)[b]	Blue	2	Orange-yellow	3
Promazine hydrochloride (Sparine hydrochloride)[c]	Blue-green	3	Orange-yellow	3–4
Promethazine hydrochloride (Phenergan)[c]	Yellow-green	2	Yellow	3–4
Trifluoperazine dihydrochloride (Stelazine)[b]	Pale green	1–2	Orange-yellow	3–4
Trimeprazine tartrate (Temaril)[b]	Bluish-green	2	Orange-yellow	3–4
Acridine dyes				
Acridine orange[d]	Orange	5	Orange*	5
Eosin-yellow[e]	Orange-red	5	Orange-red	5
Fluorescein[d]	Yellow	5	Yellow*	5
Rose Bengal[f]	Red	5	Red	5
8-methoxypsoralen[g]	Yellowish-blue	1	Yellowish-blue	3

*Slight decrease in intensity of the fluoresence.

Tested compounds from: (a) courtesy of Dr A. Kligman, University of Pennsylvania, Geigy and Fine Organics Inc., (b) Smith, Kline and French Laboratories, (c) Wyeth Laboratories Inc., (d) Chroma-Gesellschaft, Stuttgart, (e) Matheson-Coleman and Bell Co., (f) Drummond Scientific, (g) Schwarz/Mann.

Most of the tested compounds gave a phototoxic reaction, i.e. erythema or dermatitis. This reaction seemed to have no effect on the length of persistence of fluorescence.

DISCUSSION

The fluorescence of a group of photosensitizing compounds, i.e. salicylanilide, halogenated salicylanilide (HS) compounds, phenothiazine derivatives and 8-methoxypsoralen, lasted in the non-irradiated areas of the skin for 1–2 days, while the fluorescence of the acridine dyes lasted for about 5 days. After long wave ultraviolet (UVA) irradiation the fluorescence of the first mentioned group lasted longer, i.e. 3–5 days, and the colour of the fluorescence changed immediately, except in the case of 8-methoxypsoralen, which remained unchanged. The acridine dyes showed no difference in the persistence or colour of the fluorescence after UVA irradiation.

The changes observed in the first group may suggest an immediate direct effect of the UVA radiation on the fluorescent compound and/or the component(s) of the skin. This is supported by the experiment where 3,3′,4′,5-tetrachlorosalicylanilide (TCSA) showed the above-mentioned changes only when the compound was applied on the skin at the time of irradiation. Yet the irradiation of TCSA and the skin separately or simultaneously, prior to the application, did not result in any change in colour of the fluorescence.

Many of the tested HS compounds and phenothiazine derivatives have been referred to as potential photo-allergens (Hjorth & Fregert, 1968; Pathak, 1969). The action of ultraviolet irradiation on the latter has been suggested to be the formation of free radicals which bind to epidermal protein (Jenkins, Welti & Baines, 1964; Harber, Harris & Baer, 1966), or an action on the compound to produce a stable photoproduct that will act as hapten independent of further irradiation (Burckhardt, 1941; Willis & Kligman, 1968). It has been demonstrated *in vitro* that 4′,5-dibromosalicylanilide and 4′-monobromosalicylanilide are produced by UVA irradiation of 3,4′,5-tribromosalicylanilide and 4′,5-dibromosalicylanilide respectively, while 4′-monobromosalicylanilide has not been affected by further UVA irradiation (Coxon, Jenkins & Welti, 1965). On the basis of Burckhardt's theory it is suggested that in photosensitization to TBS the 4′-position, and the halogen substituted there, is a key point in the process of photosensitization (Osmundsen, 1969).

In this investigation, salicylanilide and all HS compounds produced the same changes following UVA irradiation. This may suggest that halogen atom substitutions and their position in the molecule are probably not essential for the changes observed. If these compounds are bound to the protein *in vivo*, as has been shown *in vitro* (Herman & Sams, 1971; Alani & Dunne, 1973), then the UVA radiation may possibly, through a free radical mechanism, similarly affect all HS compounds and salicylanilide to produce an unstable photoproduct, which must be in apposition to protein carrier at the time of irradiation. Or the UVA radiation may alter or activate the skin component(s), facilitating the combination with the altered or unaltered compound(s). If this holds true, would it be the case also concerning the phenothiazine derivatives, since the pattern of their changes was similar to that of HS compounds?

UVA irradiation of compounds known primarily for their phototoxic potential, i.e. acridine dyes and 8-methoxypsoralen (Hjorth & Fregert, 1968; Pathak, 1969), did not result in a change in the colour of fluorescence. However, changes in any compound could have occurred without being detected in this system, because the fluorescence of the altered compound did not emit in the visible spectrum, the spectrum of the source of irradiation did not contain the wavelength of activation of the tested compound, and/or the spectrum of the examining source did not contain sufficient energy in the excitation wavelength of the altered compound. Besides, the penetration rate and the distribution of the compound in the skin layers may be of importance in this respect.

The persistence of 8-methoxypsoralen in the irradiated area may be due to the preferred binding of this compound to DNA (Freeman, 1970), without any detectable change in the colour of fluorescence.

Although the number of observations is not large enough to permit a statistical evaluation, this study demonstrates two groups of photosensitizers; one with phototoxic properties and a second with a photo-allergic potential. The first group did not undergo any change in colour of the fluorescence in its *in situ* response to ultraviolet irradiation, whereas the second group fluoresced differently and persisted longer after irradiation.

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Effects of long wave ultraviolet radiation on photosensitizing and related compounds. II

IN VITRO BINDING TO SOLUBLE EPIDERMAL PROTEINS

MILHIM D.ALANI* AND JAMES H.DUNNE

The Photobiology Program, The Skin and Cancer Hospital, Department of Dermatology,
Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140, U.S.A.

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SUMMARY

The effects of long wave ultraviolet radiation (320–400 nm) on the binding of twenty-five fluorescent photosensitizing and related compounds to the major saline soluble guinea-pig epidermal proteins were studied. Binding before or after irradiation was detected on polyacrylamide disc gel electrophoresis by correlating the fluorescent migration pattern with the amido black counterstained pattern. Effects were seen only on photo-allergic and structurally related compounds (phenothiazines and halogenated salicylanilides). The binding of five phenothiazine derivatives was detected only after irradiation. Ten of twelve halogenated salicylanilides were bound without irradiation, and eleven were bound following it. Ultraviolet exposure appeared to increase the amount of each halogenated salicylanilide that was bound and/or to alter the existing complex.

It was previously shown (Alani, 1973) that long wave ultraviolet radiation (320–400 nm), hereafter referred to as UVA, altered the fluorescence and lengthened the persistence of potential photo-allergic compounds in the skin of mice. These findings suggested that there was an association of the compound with some portion of the epidermis. In this investigation we have studied effects of UVA on the *in vitro* binding of twenty-five fluorescent photosensitizing and related compounds (Pathak, 1969; Hjorth & Fregert, 1968) to the saline soluble proteins of guinea-pig epidermis.

MATERIALS AND METHODS

Test compounds

A 1% solution (10 mg/ml) of each compound was made in dimethyl sulphoxide (DMSO). The compounds included five phenothiazines, salicylanilide, twelve halogenated salicylanilides (HS), four acridine dyes, and three other chemically dissimilar compounds (Table 1). The 3,3′,4′,5-tetrachlorosalicylanilide (TCSA) was also used in a 1% solution in acetone and in methanol.

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* Visiting Research Instructor (Dermatology), Temple University, and Postdoctoral Fellow, Åge Bang Foundation, Denmark.

Reprint requests to Dr Alani, Axgatan 6, 431 40 Mölndal, Sweden.

Preparation of normal guinea-pig epidermal extract

The procedure was modified from the technique described by Aoki, Parker & Turk (1969). Albino Hartley-strain female guinea-pigs weighing 300–500 g were killed by ether anaesthesia. The hair was removed by plucking, and the skin was removed and stretched over a styrofoam dissecting board. The remaining hair was removed with an electric shaver. The epidermis was scraped from the dermis with a scalpel blade held perpendicular to the surface of the skin. The extraction solution (PBS) was 0·15 M NaCl adjusted to pH 7·4 by 0·01 M phosphate buffer. The epidermis was homogenized in an 'Omni-Mixer' homogenizer (Sorvall, Norwalk, Connecticut) for 10 min with a volume of PBS equal to twice the wet weight of the epidermis. The homogenizer cup was immersed in ice-water throughout the procedure. The mixture was centrifuged for 10 min at 4°C at 1740 g and the supernatant fluid saved. The remaining debris was washed with a volume of PBS equal to the wet weight of the epidermis, for fifteen washings, until the supernatant fluid was clear. The combined supernatant fluids were filtered through a Whatman No. 2 filter and then through an 0.45 μm filter to remove particulate matter. After dialysis against distilled water and the removal of additional precipitate by centrifugation, the extract was lyophilized. The powder was reconstituted as needed to 5 mg/ml in PBS.

Preparation of epidermal extract (EE) and test compounds for polyacrylamide disc gel electrophoresis

The following samples were prepared: 1 ml of EE; 1 ml of EE with 20 μl DMSO added; 1 ml of EE with 20 μl 1% DMSO solution of each compound; 1 ml PBS with 20 μl 1% DMSO solution of each compound (control sample). Each sample was stored overnight at room temperature in the dark. Half of each sample was saved for electrophoresis. The other half was placed in a 1 mm path quartz spectrophotometer cuvette and irradiated with UVA for 5 min with a xenon arc solar simulator, utilizing a Schott filter No. 345_2 (formerly WG3, 2 mm thick), which filters out wavelengths less than 320 nm (Berger, 1969). The approximate ultraviolet dose delivered to the facing surface of the cuvette was 1 J.cm^{-2}. All the samples were analysed by electrophoresis within 1 h after completing irradiation.

Polyacrylamide disc gel electrophoresis

A modification of the procedure described by Davis & Ornstein (1971) was used. Each sample to be separated consisted of 0.075 ml of the test solution and 0.025 ml of 40% sucrose. The sample was gently layered between the buffer and the stacking gel just prior to electrophoresis. A 2 mA initial current was applied to each column to prevent conduction loss into the buffer. After 15 min the current was increased to 4 mA per column. Riboflavin, which was used as a catalyst for the stacking gel, served as the tracking dye. It was brown when observed in visible light, and fluoresced yellow when seen in ultraviolet radiation. The columns were not examined for fluorescence until the electrophoresis was complete. The fluorescent pattern was observed with a 'Mineralight UVSL 25' (Ultraviolet Products, San Gabriel, California). The intensity of the fluorescence of the column containing unirradiated sample was compared simultaneously with the column containing the irradiated sample of the same compound. A subjective assessment of the relative fluorescence intensity of the paired columns was made independently by each of the authors. Immediately after observing the fluorescence, the columns were fixed in 7·5% acetic acid. The distance of the fluorescent bands and the tracking dye from the edge of the separating gel was measured after fixing. Following counterstain with amido black, the protein separation pattern was measured and correlated with the fluorescent pattern.

RESULTS

Epidermal extract

Immediately after the completion of electrophoresis, the only visible fluorescent band was the riboflavin dye (Rf 1.0) at the distal end of the columns. Fifteen protein bands were seen in the amido black stained columns. Band 8 (Rf 0.52) stained much darker than all the other bands, and contained most of the protein in the sample. Neither DMSO nor UVA irradiation altered the amido black separation pattern.

TABLE 1. Polyacrylamide disc gel electrophoresis: the binding of twenty-five fluorescent compounds before and after ultraviolet irradiation (UV) to the major saline soluble epidermal protein (EE)

Tested compound	Binding to EE* (Rf 0.52)		Unbound chemical in EE or phosphate buffered saline (Rf 1.0)	
	Without UV	With UV	Without UV	With UV
Phenothiazines				
Chlorpromazine hydrochloride (Thorazine)[a]	o†	+ ‡	+	+
Prochlorperazine edisylate (Compazine)[a]	o	+	+	+
Promazine hydrochloride (Sparine)[b]	o	+	+	+
Trifluoperazine dihydrochloride (Stelazine)[a]	o	+	+	+
Trimeprazine tartrate (Temaril)[a]	o	+	+	+
Salicylanilide and halogenated salicylanilides[c]				
Salicylanilide	o	o	+	+
3,3′,4′,5-tetrachloro-(TCSA)	+	+ + §	+ **	+ **
3,3′-dichloro-	+	+ +	+	+
3′,4′-dichloro-	+	+	+	+
3,5-dichloro-	+	+ +	+	+
4′,5-dichloro-	+	+ +	+	+
3-chloro-	o	+	+	+
3′-chloro-	o	o	+	+
5-chloro-	+	+ +	+	+
3,4′,5-tribromo-(TBS)	+	+ +	+ **	+ **
4′,5-dibromo-	+	+ +	+	+
4′-bromo-	+	+ +	+	+
5-bromo-	+	+ +	+	+
Acridine dyes				
Acridine orange[d]	+	+	+	+
Rose bengal[e]	+	+	+	+
Fluorescein[d]	o	o	+	+
Fluorescein isothiocyanate[f]	+	+	+	+
Other compounds				
Demethylchlortetracycline (Declomycin)[g]	o	o	+ **	+ **
8-methoxypsoralen[h]	o	o	o	o
Protoporphyrin[h]	o	o	+	+

* No fluorescent band was seen at Rf 0.52 in any of the non-irradiated or irradiated PBS controls.

† No detectable binding.

‡ Binding to major epidermal protein which was seen as fluorescent band.

§ Increased binding to the same band which was seen as increased intensity of the fluorescent band.

** See text.

Tested compounds obtained from: (a) Smith, Kline and French Laboratories, (b) Wyeth Laboratories Inc., (c) Courtesy of Dr A. M. Kligman, University of Pennsylvania, and Geigy, (d) Chroma-Gesellschaft, Stuttgart, (e) Drummond Scientific, (f) Aldrich Laboratories, (g) Lederle Laboratories, (h) Schwarz/Mann.

Phenothiazine derivatives

None of the phenothiazine derivatives (Table 1) could be seen migrating with any protein band in the non-irradiated samples. After UVA irradiation, a yellow fluorescent band migrated with band 8 (Rf 0.52) in all five of these compounds, and no similar band was seen with the irradiated PBS controls. All of the unbound chemicals migrated with riboflavin in EE and PBS control samples.

Salicylanilide and the halogenated salicylanilides

The results are listed in Table 1. In the non-irradiated samples of the chemical plus EE, ten of twelve HS could be seen as a blue fluorescent band migrating in the middle of the gel column. This band corresponded with protein band 8 (Rf 0.52) in the amido black counterstained columns. Eleven of twelve UVA irradiated samples migrated with protein band 8, and the apparent intensity of the fluorescent band was increased in all. Salicylanilide itself did not migrate with any protein band, before or after irradiation.

A bluish fluorescent band, which was interpreted as unbound chemical, was seen migrating with the riboflavin in all samples of the phenothiazines and HS, in either EE or PBS controls. Unbound chemical was also seen as two other fluorescent bands, located between band 8 and riboflavin in each of the non-irradiated EE and PBS samples containing TCSA and 3,4',5-tribromosalicylanilide. These two bands were present in decreased intensity in the irradiated samples.

TCSA changed the protein separation pattern. Band 8, although normal in the non-irradiated sample, was widened distally in the irradiated specimen to include band 9. Band 14 stained darker than normal in the non-irradiated sample, but almost disappeared in the irradiated sample. The fluorescent and amido black stained patterns of non-irradiated and irradiated samples were not altered by the different solvents used for TCSA, i.e. DMSO, methanol, or acetone.

Acridine dyes and other compounds

The results are summarized in Table 1. Acridine orange, rose bengal, and fluorescein isothiocyanate without irradiation, were bound to band 8; UVA irradiation had no effect on the fluorescent pattern. Protoporphyrin, fluorescein, and demethylchlortetracycline did not migrate with any protein, nor was any effect of UVA exposure seen. Unbound chemicals of this group of compounds migrated with riboflavin in EE or PBS. Unbound demethylchlortetracycline migrated between band 8 and riboflavin, and with riboflavin in EE or PBS.

DISCUSSION

This investigation demonstrates that long wave ultraviolet radiation (320–400 nm: UVA) has an effect on the binding of phenothiazines and halogenated salicylanilides (HS) to saline soluble epidermal proteins. The binding was detected as a fluorescent band that migrated with the major soluble epidermal protein band in polyacrylamide disc gel electrophoresis.

It appears that UVA irradiation is necessary to establish a phenothiazine–protein bond. On the other hand, most of the HS were bound with the major soluble epidermal protein without any irradiation and, following UVA exposure, the intensity of the fluorescence was increased. These results differ from those of Herman & Sams (1971), who were unable to detect binding of TCSA to guinea-pig albumin unless the sample was first irradiated by UVA. Since they used a different protein (guinea-pig albumin rather than EE) and a different solvent (ethanolic phosphate buffered saline rather than PBS with no added ethanol) their results are probably not directly comparable to this study. No observable effect of UVA exposure was seen on any of the remaining compounds. These were either bound without UVA exposure (i.e., acridine orange, rose bengal and fluorescein isothiocyanate) or remained unbound after UVA irradiation (i.e., fluorescein, protoporphyrin, 8-methoxypsoralen and demethylchlortetracycline).

Any of the compounds could have been bound with or without UVA exposure and remain undetected by this technique, due to one or more of the following possibilities: quenching in the system; inefficient fluorescent characteristics of the chemical–protein complex; a fluorescent emission spectrum of the complex not in the visible spectrum; or the spectrum of the 'Mineralight' not containing sufficient energy in the excitation wavelength of the complex.

Several possible effects of UVA on HS and/or protein must be considered.

(1) UVA may quantitatively increase the amount of fluorescent material which is bound to the protein. This newly bound material may represent the original compound or any of its photodegradation products, which may be formed during UVA irradiation.

(2) UVA may alter the structure or configuration of the chemical–protein complex without actually increasing the amount of the chemical which is bound. Such changes could alter the fluorescent characteristics of the complex and result in the increased intensity of the fluorescence.

(3) Finally, a combination of the above may occur, i.e. increased binding plus a change in the structure or configuration of the original compound as a result of UVA irradiation.

Coxon, Jenkins & Welti (1965) have shown by infrared spectrometry that halogen atom substituents on the salicyl ring of salicylanilide anions in basic ethanol solution are replaced by hydrogen atoms as a result of near ultraviolet irradiation. The chlorine atom in the 5-position, and bromine atoms in the 3- or 5-position are replaced by hydrogen atoms, while the 5-chlorine and all halogen substituents on the anilide ring are unaffected. As predicted from the results of Coxon *et al.* (1965), all HS with 3- or 5-bromo or 3-chloro substituents showed increased binding to EE protein following UVA irradiation. In addition, however, 5-chloro and 4′-bromo substituted HS also demonstrated UVA-induced binding. Only salicylanilide and the 3′-chloro substituted HS did not bind to protein, while the binding of the 3′,4′-chloro substituted anilide ring was unaffected by UVA. In experiments with mouse skin, all the HS studied appeared to bind to epidermal components following UVA exposure (Alani, 1973), in spite of apparent unreactivity in EE or basic ethanol solution.

The photosensitizing compounds studied here can be divided into two groups; those in which an effect of UVA is observed on binding to soluble epidermal protein, and those in which no effect of UVA is seen. The former (HS and phenothiazines) are known photo-allergens or are structurally related to known photo-allergens. The latter (acridine dyes, porphyrins, demethylchlortetracycline, 8-methoxypsoralen and fluorescein) are usually considered to be only phototoxic.

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Evaluation of skin damage. I

SKIN RESISTANCE MEASUREMENTS WITH ALTERNATING CURRENT (IMPEDANCE MEASUREMENTS)

F.A.J.THIELE AND K.E.MALTEN

Department of Dermatology, University of Nijmegen, Javastraat 104, Nijmegen, The Netherlands

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SUMMARY

The impedance of human skin was measured at a frequency of 25 Hz and 0·032 mA before, during and after exposing 6 cm^2 skin for 60–240 min to 10 or 12 ml of alkali (NaOH and sodium phosphate) and sodium chloride solutions, adjusted to different pHs. From the impedance and phase angle values, measured at intervals of 10–60 min between different pairs of skin sites, the changes in the single transition resistances of these skin sites were calculated.

Sodium hydroxide (NaOH) solution at pH 12·0, within 10 to 15 min, abolished skin resistance completely; a solution of sodium phosphate, adjusted to the same pH, affected skin resistance similarly, but only after 60 minutes.

The order of severity in skin effects of solutions of other alkaline substances, adjusted to different pHs was determined.

Most studies of irritation have focused on the skin's morphological appearance; more quantitative and convenient objective methods are needed to determine functional abnormalities in incipient dermatitis. Transepidermal water loss measurements fit such a requirement (Spruit, 1970, 1971; Thiele & Horst, 1967). In a search for other routine measurements, attention was directed towards the resistance of skin to an alternating current (impedance measurements) (Allenby *et al.*, 1969; Rosendal, 1944; Tregear, 1965). Although skin impedance measurement *in vivo* is physiologically more difficult to understand, it may have the advantage that it can show the damaging effect quantitatively during the exposure to the irritant (Salanski & Nezval, 1968; Schutter, 1969, 1970).

The resistance (R) of skin to an alternating current (skin impedance) is defined as the (transfer) impedance value or magnitude (Z) divided by the cosine of the phase angle F (cos F), being dependent on the particular frequency (Hz) and amperage (mA) applied: $R = Z/\cos F$. Hazewinkel (1971) and Hazewinkel & Lubbers (1971) claimed that they can possibly differentiate *in vivo* the resistance of the horny layer from that of the sweat glands by varying the frequencies and amperages at which measurements are performed. The experimental circumstances used here lead to results which are related to changes in the properties of the horny layer (Yoshida *et al.*, 1971) and sweat glands. Our approach, deliberately empirical, aims to investigate whether skin impedance measurements could be used to determine at an early stage (during the exposure itself) whether externally applied water soluble substances influence the skin to such an extent that changes in freedom of movement of water and ions occur, manifesting themselves by variations in resistance.

MATERIALS AND METHODS

Apparatus

Radiometer Impedance Meter, type GB 11^c *(Radiometer/Copenhagen)*. This meter enables the measurement of impedance (Z) up to $10^6\ \Omega$, in a frequency range from 25 to 100 kHz. Impedance (Z) and phase angle (F) can be read directly.

Routine measurements were carried out at a frequency of 25 Hz and 0·032 mA; at high skin resistances 0·32 mA was used.

Platinum electrodes were used, which consisted of a horizontal platinum plate (1 cm^2) with a soldered wire, insulated by a hard PVC tube, passing through a rubber stopper at one side of which a flexible thermistor (rectal thermistor, Yellow Spring) could be introduced for measuring the temperature of the test solutions during the experiment. The electrodes, provided with insulated wires and plugs for connecting to the impedance meter, are marked K (1), L (2), M (3) and N (4), sequentially dependent on the number of electrodes in use.

Perspex skin cups, covering a skin surface area of 6·4 cm^2 and marked in a similar fashion, were provided with metal rings with slits and Velcro strips for convenient attachment to the volar aspect of the forearm. The cups could be closed with perspex lids or with the electrode-supporting rubber stoppers.

Performance of measurements

On the volar aspect of one or both forearms of a test subject, three or four measuring sites were marked K, L, M and N respectively from the wrist upwards. The site K on each forearm was about 6 cm proximal to the wrist, and the distance between the midpoints of the measuring sites was about 4·5 cm. The perspex cups were attached to these marked skin areas and filled with 10 ml of 0·015 M NaCl solution. The cups were closed with the rubber stoppers pierced by the electrodes (see exposure scheme I). After 5, 15 or 30 min contact between the skin and salt solution, the electrodes were connected to the impedance meter and the first measurements made. Impedance (Z values in ohms) and phase angle (F values in degrees) readings were taken from any combination of two cups. On carrying out measurements with three cups, these consisted of readings between K–L, K–M and L–M (Fig. 1), whereas with four skin cups, six values for Z and F each were matched, i.e. between K–L, K–M, K–N, L–M, L–N and M–N (Fig. 2). The readings were recorded as Z K–L for the impedance between K

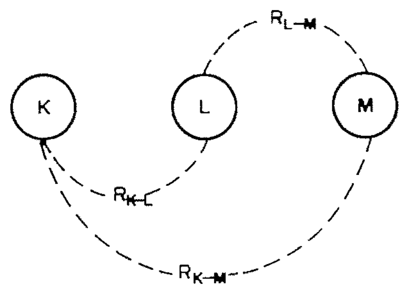

FIGURE 1. Scheme for measuring impedance at three skin sites on the forearm.

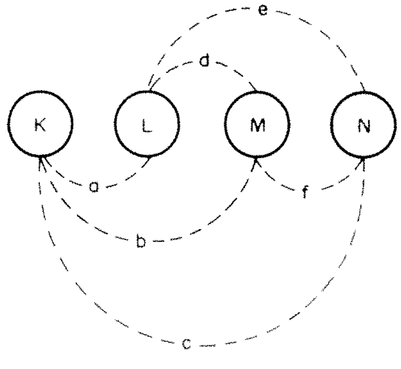

FIGURE 2. Scheme for measuring impedance at four skin sites on the forearm.

and L and the phase angle recorded as F K–L, etc. The negative or positive positions of the phase angles were measured. The values obtained after 15 or 30 min contact with NaCl were used as the blanks for the measuring sites.

After the blank measurements, the cups were emptied and then filled with the test solutions of known composition and pH; 10 or 12 ml in each cup. At least one or two cups on one or both forearms were refilled with 0·015 M NaCl solution, since they served as the blank references in case any unexpected drift in the blank occurred during the experiment. After 30, 60, 90 or 120 min (or longer) the readings of Z and F for any combination of two cups were repeated.

After the last measurement on the test solution, the cups were emptied again, the skin surface, cups and electrodes were thoroughly rinsed with the 0·015 M NaCl solution, and the cups were subsequently refilled with this solution. After replacing the electrodes in the skin cups, the Z and F measurements were repeated and recorded as 'end values'. If measurements were carried out on four areas of both forearms, a maximum of six solutions could be evaluated simultaneously, since at least two areas continuously served as blank NaCl references.

Even though each experiment was not designed according to the Latin Square Method (only the measuring sites 2 and 4 are interchanged with the duplicates), statistical analysis of the numerical results makes it clear that the measuring sites were not significantly different. This conclusion, already presented for water loss measurements after skin exposures to detergent (Stephens, 1963), was reported by Schutter (1969), after reviewing his data obtained during a period of about 3 years with the measuring technique described in this communication.

Calculation

From the impedance/phase angle combinations, the resistance value R can be calculated, according to $R = Z/\cos F$. It is assumed that the Z-values of the skin consist of a capacitance with a parallel resistance. If two measuring sites were exposed to test solutions with unknown properties, and a third site to the blank reference NaCl solution (0·015 M); three Z and three F values could be read from these three R values may be calculated (Fig. 1.) Each of these resistances is composed of two single transition resistances (skin–NaCl–electrode). So R_{K-L} is composed of $R_K + R_L$, whereas R_{K-M} and R_{L-M} are composed of $R_K + R_M$ and $R_L + R_M$, respectively.

Mathematically it can be derived that:

$$R_K = \tfrac{1}{2} R_{K-L} + \tfrac{1}{2} R_{K-M} - \tfrac{1}{2} R_{L-M}$$
$$R_L = \tfrac{1}{2} R_{K-L} - \tfrac{1}{2} R_{K-M} + \tfrac{1}{2} R_{L-M}$$
$$R_M = -\tfrac{1}{2} R_{K-L} + \tfrac{1}{2} R_{K-M} + \tfrac{1}{2} R_{L-M}$$

Thus $R_K + R_L = R_{K-L}$, etc.

It implies that R_x, the single transition resistance of one measuring site, can be calculated from the resistance values derived from the Z and F values for three measuring sites.

If four measuring sites were exposed to three different test solutions and one NaCl solution, six values for Z and F could be read (a–f) (Fig. 2).

All calculations were performed on a computer, programmed in Fortran IV. Since there are only four unknowns (the R-values of K, L, M and N: the single transition resistances of four measuring sites), the computer is programmed so that four equations are used for calculating these four unknowns, although six measurements for Z and F are routinely taken, giving six equations. By using different combinations of four equations (i.e. a+b+c+d or a+b+c+f, etc.), the results of the measurements and of the computer calculations can be routinely checked against each other.

The print out of these calculations are given in the tables as (−RED) and (Pct RED). The values under (−RED) are the delta R-values (single R transitions for each measuring site) with respect to the measured NaCl blank references (in $\Omega \times 10^3$). If the delta R-value is printed positive the resistance has decreased, whereas a negative printed delta-value under (−RED) indicates an increase in the resistance relative to the blank. The Pct RED is the residual resistance as a percentage of the initial blank NaCl values of each of the sites measured.

Since the computer is unable to print the phase angle in degrees, these degrees are recalculated by the computer in radians. $180° = 180/3{\cdot}14159 = 57{\cdot}2975$. Thus $R = Z/\cos F$ becomes $R = Z/\cos F/57{\cdot}2975$.

Graphs

The Pct RED (residual resistance) values may be plotted against the time-sequence of the measurements. It is more informative if the percentage decreases in skin resistance (100 − Pct RED) are plotted against time. The curves obtained by connecting these points are designated 'skin resistance curves'. At 100% the resistance of skin has reached zero. Corrections on the test area values for the occasionally observed drift in the R-values occurring on contact with NaCl solutions (used as blank references) can be carried out as well, resulting in 'delta skin resistance curves'.

Experimental design

Measuring conditions and measuring areas. The measurements were carried out on the volar aspects of the left and right forearms of male and female subjects, after a pre-conditioning period of relatively low physical activity of about 1 h in a temperature controlled room.

Compositions of the test solutions (solutions of exposure scheme no. 1). The compositions and pHs of the solutions under test were as follows: the phosphate solutions were Na_3PO_4 0·002 M, the pHs of which were adjusted with a few drops of concentrated NaOH solution to 10·0 or 12·0 (controlled by electrical pH measurement).

RESULTS

Exposure scheme no. 1

The changes in skin resistance on exposing skin sites of left and right forearms for 1 h to solutions of NaOH and Na_3PO_4, adjusted to different pHs, are shown in Table 1 and Figs. 3–5. The NaCl references are given by the broken lines in Fig. 3b. From these data it could be seen that:

NaOH (pH 12) solution decreased skin resistance (R) almost completely within 10 min (90%) (Fig. 3, curve a); after 30 min a decrease of 100% was measured. After 60 min contact, intense inflammation with redness, maceration, papules and vesicles was observed. The vesicles disappeared

EXPOSURE SCHEME NO. 1. Prior to and after each specific exposure, the skin was exposed for 15 min to 0·015 M NaCl. On the O marked skin areas no specific exposure was carried out. Instead of filling these cups with the specific solutions under test they were filled with 0·015 M NaCl (= 0·09%) as a reference

Left forearm	Test area	Right forearm	Total no. of exposures	Duration of first and successive daily exposures	Figures 4–5–6 (curves)
NaOH (pH 12)	N = (4)	O	6	60 min	(a)
O	M = (3)	Phosph. (pH 12)	6	60 min	(b)
NaOH (pH 10)	L = (2)	O	6	60 min	(c)
O	K = (1)	Phosph. (pH 10)	6	60 min	(d)

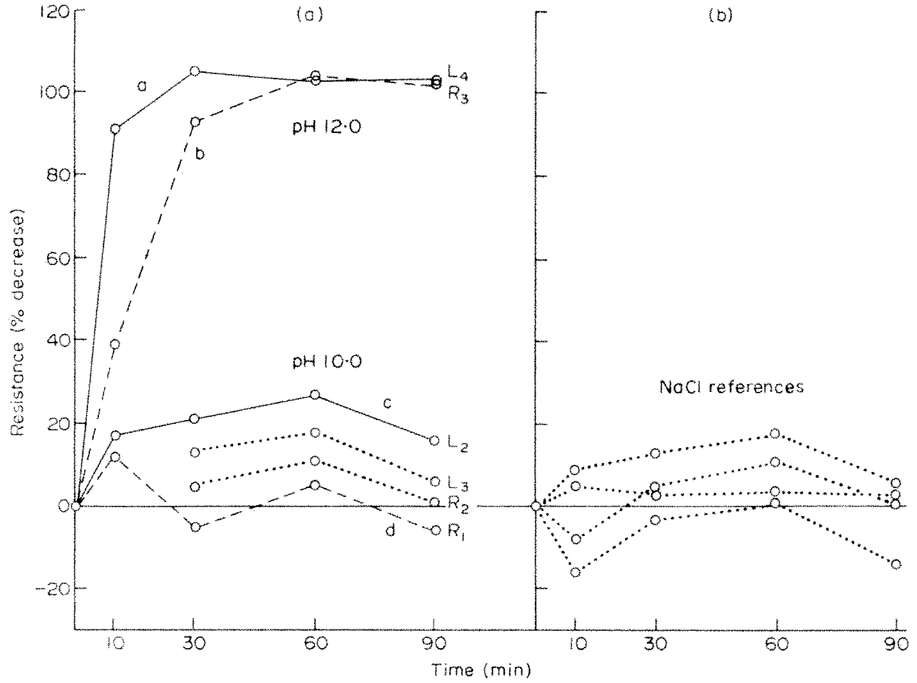

FIGURE 3. Exposure scheme 1. (a) Skin resistance versus time: curves obtained by exposing skin for 60 min to NaOH (pH 12) (curve a), Na_3PO_4 (pH 12) (curve b), NaOH (pH 10) (curve c), and Na_3PO_4 (pH 10) (curve d), followed by exposing the four skin sites for 30 min to a 0·09% NaCl solution. (b) Skin resistance versus time: curves obtained by exposing four skin sites for 60 min to a NaCl solution (0·09%). After 60 min, the cups on left and right forearms were emptied and refilled with a fresh NaCl solution for the following 30 min.

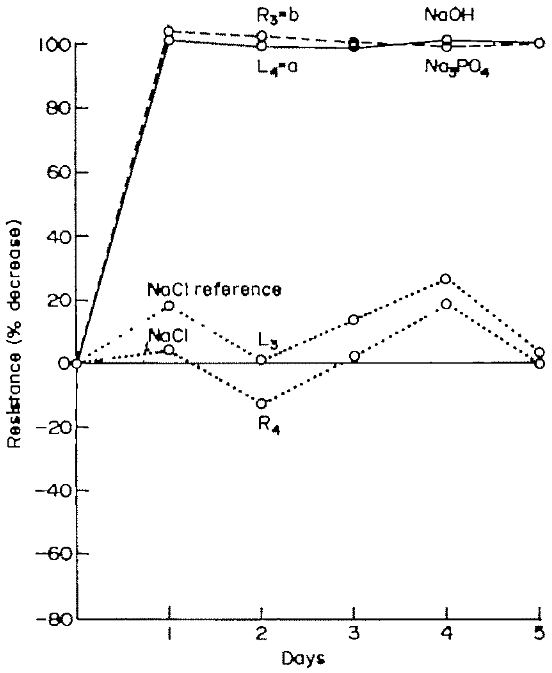

FIGURE 4. Exposure scheme 1. 60 min values. Skin resistance versus time: curves by connecting the daily measured 'end values' for skin resistances after exposing skin to NaOH (pH 12) (curve a, skin site L_4), Na_3PO_4 (pH 12) (curve b, skin site R_3), and NaCl (0·09%) solutions (skin sites L_3 and R_4).

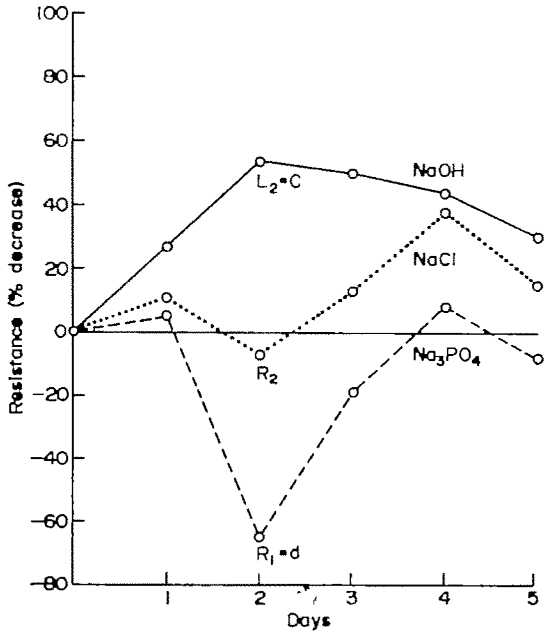

FIGURE 5. Exposure scheme 1. 60 min values. Skin resistance versus time: curves obtained by connecting the daily measured 'end values' for skin resistance after exposing skin to NaOH (pH 10) (curve c, skin site L_2), and Na_3PO_4 (pH 10) (curve d, skin site R_1). On the sixth day the skin sites were all exposed in an identical fashion. However, no skin resistance measurements were carried out on that day.

TABLE 1. Results of impedance measurements

	Resistance (% decrease)				
	1st day	2nd day	3rd day	4th day	5th day
NaOH (pH 12·0)					
Recuperated starting point		87	102	102	101
10 min	91	100	99	99	100
30 min	105	100	98	100	100
60 min	103	99	101	101	100
Na_3PO_4 (pH 12·0)					
Recuperated starting point		66	105	102	107
10 min	39	109	101	100	102
30 min	93	102	101	100	101
60 min	104	102	100	99	100
NaOH (pH 10·0)					
Recuperated starting point		16	21	51	−6
10 min	17	47	64	58	24
30 min	21	52	61	46	27
60 min	27	54	50	44	30
Na_3PO_4 (pH 10·0)					
Recuperated starting point		−44	−60	−27	−71
10 min	12	−88	−27	−3	−26
30 min	−5	−70	−18	1	−17
60 min	5	−64	−19	8	−8

after 60–90 min. The following day tiny brown crusts were observed. Daily repeated exposures on the same skin site gave no further alteration: the 100% decrease persisted (Fig. 4, curve a).

Sodium phosphate (pH 12) solution decreased skin resistance (R) about 40% within 10 min (Fig. 3, curve b); after 30 and 60 minutes the values for the decrease in R approached 90 and 100% respectively. The inflammation which developed on this skin site was clinically less severe in comparison to the NaOH (pH 12) exposed skin site: no vesicles or papules were observed.

Differences in effect between NaOH (pH 12) and Na_3PO_4 (pH 12) could also be seen from the values of the recuperated resistance, about 24 h later, before re-exposure. They were then 13% (100–87%) and 34% (100–66%) respectively (Table 1), thus demonstrating a difference in capacity for recovery in the first 24 h after exposure. On repeated exposures, the 100% decrease in R persisted (Fig. 4, curve b) and no recuperation manifested itself on the third, fourth or fifth day (Table 1).

NaOH (pH 10) solution decreased skin resistance on first exposure by about 15% within 10 min; the end value (day 1) was similar (Fig. 3, curve c). The second exposure further decreased R (Fig. 5, curve c). However, after the exposure on the third day the resistance was less markedly decreased. This trend progressed until after the exposure on the fifth day; end resistance of 70% 'adaptation'. The recuperation value on this last day showed a slight increase in R (Table 1) over the initial value of the first day.

Sodium phosphate (pH 10) solution influenced skin resistance during the first exposure to a minor extent (Table 1 and Fig. 3, curve d). Subsequently, a tendency to increasing resistance was observed: just before the second exposure the resistance was increased by 44% and an increase in resistance of about 64% could be measured after this second exposure (Fig. 5, curve d). This same tendency continued during the next days.

DISCUSSION

Allenby *et al.* (1969) established by *in vitro* experiments that the impedance of skin is localized in the horny layer. At low frequencies the impedance approximates the direct current resistance and polarization is not a serious problem. Impedance, in this case, is largely determined by the resistance component of skin (Hazewinkel & Lubbers, 1971, 1973; Tregear, 1965), and this may be expected to correlate with ion mobility in the skin barrier. Nevertheless, impedance measurements *in vivo* can represent the properties of the whole 'surface sheath' including appendages (Rosendal, 1943, 1945), which form a sharp electrical boundary with the cutis, the capillary bed and the more highly vascular tissues (muscles) whose impedances are known to be relatively low (Barnett, 1938; Cole, 1932; Gunn & Curran, 1971; Lullies & Rumberger, 1966; Rosendal, 1944; Schutter, 1970; Spruit, 1971; Stephens, 1963).

The results reported here show that skin impedance measurement *in vivo* has the advantage that it can show quantitatively the damaging effect during exposure to the irritant.

From the graphs of skin resistance curves it can be seen that certain water soluble substances can produce decrease or increase in skin resistance either slowly or rapidly. From the total experimental evidence the following conclusions seem warranted.

The 90 (60+30) min exposure of skin on the volar aspect of the forearm of an apparently normal healthy subject to a 0·09% solution of NaCl (10 ml/6 cm^2) scarcely influences the skin resistance (Fig. 3b). Daily fluctuations may reflect normal physiological variations in the electrical properties of the skin.

The 60 min exposures to solutions with a pH of 10·0 (NaOH and Na_3PO_4), carried out in a similar fashion, again scarcely influence the skin resistance (Fig. 3a).

If the pH of these solutions is increased to 12·0 (by adding 0·02 M NaOH), they abolish skin resistance completely.

If the 60 min exposures to NaOH (pH 12) and Na_3PO_4 (pH 12) were repeated on successive days, skin resistance remained abolished (Fig. 4). Some recuperation from the first damage inflicted could be registered only about 24 h after that first exposure: the resistance values appeared to be 13 and 34% of the initial blank values, measured on the first day, before the exposures were carried out. On subsequent days there was no recovery apparent. Repetition of the daily exposures to NaOH (pH 10) and Na_3PO_4 (pH 10) solutions showed less dramatic effects (Fig. 5 and Table 1). The initial progressive decrease in resistance by NaOH (pH 10) was followed by some slight increase after the exposures on the third and fourth days. This may be comparable with what Malten & Spruit (1966) described as 'adaptation' after carrying out a similar experiment in which, however, the increased water-vapour loss (measured with the electrolytic water meter: Meeco) was used as a parameter. It must be kept in mind that in our experiments a 6 cm^2 skin area was exposed to about 10 ml of a pH 10 solution, whereas in the 1966 experiment a 2 cm^2 skin area was exposed to only 1 ml of a NaOH solution at pH approximately 12·0. The Na_3PO_4 (pH 10) solution was able to increase skin resistance: on the second day this increase was about 64% above the initial blank value. However, the successive exposures could not maintain this high 60 min value of skin resistance (Fig. 5 and Table 1).

The starting values as measured about 24 h after the foregoing exposure and just previous to the following exposure, of both solutions, however, showed a weak [NaOH (pH 10)] or a stronger tendency to recover (Table 1), even showing some 'overshoot' [Na_3PO_4 (pH 10)]. As a consequence of the repeated Na_3PO_4 (pH 10) exposures, an increase in skin resistance was built up.

No measurements were carried out to determine whether the decreases in skin resistance are concentration and/or time dependent. However, in a communication presented at a University

Meeting (Department of Dermatology, University of Nijmegen, The Netherlands, May 1970), Schutter (1970) reported the following conclusion.

The statistician experimentally set up the following equation: pCt R = a+bC+cT, in which C was the concentration of the solution under test (detergent solutions) and T the exposure time. It was possible to determine the constants (a), (b), and (c) from the mean values obtained during his measurements, and to replace them by numerical values. The equation for the particular detergent component under test became pCt RED = 95·3 − 0·18 C + 29·05 T, in which all the means of the residual resistance fitted. So the percentage residual resistance is a linear function of the concentration and the exposure time.

Since *in vitro* experiments have shown a good correlation of impedance with water permeability (Allenby *et al.*, 1969), and since we have information available about the influence that chemicals may have on water loss *in vivo* (Rollins, 1968; Spruit, 1970, 1971; Thiele & Horst, 1967), the results of our impedance measurements *in vivo* were compared with water loss and carbon dioxide release measurements performed on the same skin sites, *in vivo*.

Extension of this work to other possibly toxic compounds (soaps, detergents, washing powders, proteolytic enzymes, etc.), which are outside the scope of this paper, as it is primarily concerned with presenting a new methodology, has been carried out and will be communicated separately (Malten & Thiele, 1973; Thiele & Malten, 1973).

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A scanning electron microscope study of the effects of uniaxial tension on human skin

IAN A. BROWN

Bioengineering Unit, University of Strathclyde, Glasgow

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SUMMARY

The effect of progressive extension on the structure of human skin has been studied by scanning electron microscopy. Varying degrees of strain were imposed on abdominal skin specimens by a carefully controlled mechanical testing technique. Progressive straining has a two-stage effect on the epidermis: initial extension pulls it into a flat layer, and further extension elongates the cells. The dermal fibres reorientate, straighten, become aligned and compacted, in response to increasing strain. The onset of these changes varies through the thickness of the dermis. The effects of strain on hair follicles, glands and fat cells are described. The structural deformations are related to the mechanical behaviour of the skin, and clinical implications of the findings are discussed.

Studies of the structural distortions produced in normal human skin by stretching may give insight to the biological effects of prolonged tension caused by oedema or certain reconstructive plastic surgical operations. Further, by relating structural deformations to mechanical behaviour, the reasons may be indicated for the abnormal mechanical function which characterizes certain pathological conditions of the skin (e.g. Ehlers-Danlos syndrome). This paper describes a scanning electron microscope study of the effects of progressive straining on the structure of abdominal skin.

Previously, optical microscope studies of mechanically deformed skin were concerned with the sequence of changes occurring in the dermal fibre network in response to progressive straining (Lee, 1957; Craik & McNeil, 1965; Ridge & Wright, 1965), and with showing how these changes relate to the non-linear load–extension behaviour of human skin (Gibson, Kenedi & Craik, 1965; Ridge & Wright, 1965). A typical load–extension graph is shown in Fig. 1. The non-linearity was explained in terms of three phases of extension: during the initial phase of great extension at low load, the dermal fibres reorientate towards the load axis and become straighter; in the second phase, characterized by increasing stiffness, the fibres progressively become aligned, and come directly under load; extension of the fibres themselves produces the almost linear third phase. Normal physiological activity causes strains in the first half of the second phase (Gibson, Kenedi & Craik, 1965). Extension of the skin beyond the third phase initiates rupture of the skin by a yielding process, which is thought to be due to the progressive breaking of individual fibres rather than fibre slippage (Craik & McNeil, 1965). No attention was given in these studies to the effect of mechanical strain on the non-fibrous

Present address: Textile Research Institute, PO 625, Princeton, New Jersey 08540, U.S.A.

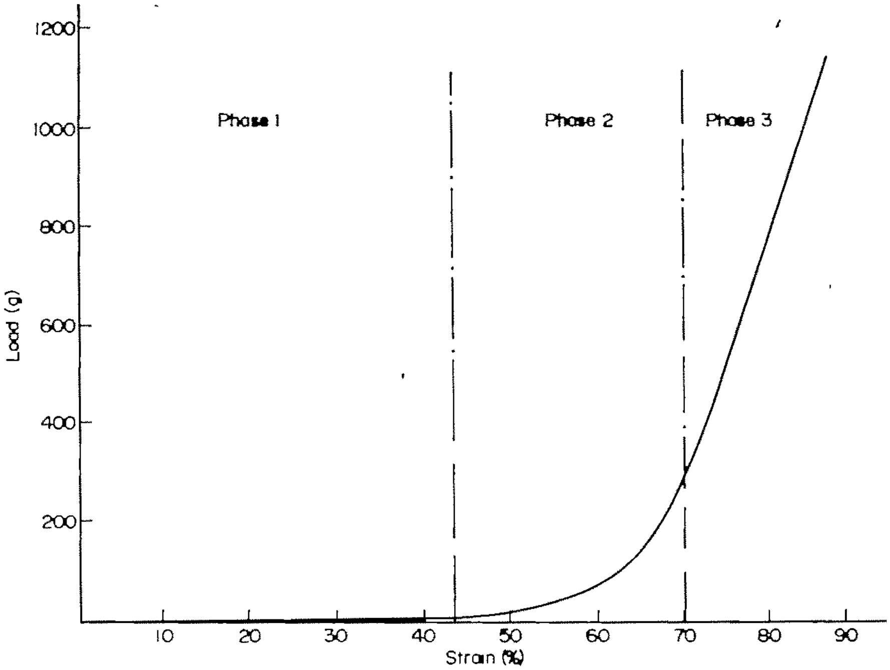

FIGURE 1. A typical load strain graph obtained in an *in vitro* uni-axial test of abdominal skin. The three phases of extension are indicated. Maximum strain level shown is below the yield point. Specimen width, 8 mm.

components of the skin, and fine details were not studied, because of the limitations of optical instruments.

Recent scanning electron microscope (SEM) studies have shown the efficacy of this technique for determining the internal morphology of the human skin, through high resolution three-dimensional visualization. Cells, hair follicles, glands, and blood vessels, have been identified (Millington & Brown, 1970). Studies of the dermal fibres have described their arrangement and structure through the thickness of normal skin (Brown, 1972), and gross changes in fibre form in pathological skin (Dawber & Shuster, 1971). SEM studies of the effect of mechanical strain on skin structure have been of a preliminary nature (Finlay, 1969; Carr & Millington, 1969). The mechanical techniques used in these studies did not incorporate controls over the condition of the specimen environment during test, or of of the rate of straining; such controls are required for meaningful mechanical tests of the skin (Daly, 1966). Microscopic observations were restricted to the dermal fibres. The purpose of this study was to use the SEM to examine the effects of progressive mechanical straining, imposed under controlled conditions, on the various structural components of the skin, and to relate these changes to the level of the applied strain.

MATERIALS AND METHODS

Skin samples from twelve adults were obtained prior to autopsy from the abdomen, in the region midway between the xiphisternum and the umbilicus. A brass frame (75 × 75 mm) was attached by an adhesive to the skin before excision to prevent stretching the skin during the cutting process. The orientation of the sample relative to the body axes was recorded by markings on the frame. On removal from the body the specimen was wrapped in polythene to prevent exsiccation, enclosed in an air-tight container and stored in a refrigerator at 4 °C before mechanical testing within 48 h of the time of death.

Preparation of specimens for mechanical testing involved careful excision of subcutaneous fat, detachment from the frame and cutting of the sample, by a cutting die, into mechanical test specimens 8 mm wide. Up to seven specimens lying side-by-side could be obtained from one sample. Specimens were cut parallel to the cranio-caudal meridian. A fully relaxed specimen was put in fixative at this point, and the remainder stored in Ringer's solution prior to mechanical testing.

Mechanical tests were performed on an Instron Tensile Testing Machine (Floor Model TT-C) with a glass tank mounted on the cross-head. Specimens were mounted on the Instron with the aid of a device which ensured a standard test length, minimized manipulation of the specimen, and enabled the specimen to be held between the grips, at the start of the test, in a fully relaxed condition. The immersion tank was filled with circulating Ringer's solution maintained at 37 °C to provide a uniform test environment for each specimen.

In each group, specimens were strained at a constant rate to discrete load levels corresponding to successive points on the load extension graph. At the desired load level the cross-head was stopped and the specimen allowed to stress relax for 15 min. A fixation device was attached to the specimen clamps to maintain the skin in the stretched condition on removal from the Instron. The complete clamp assembly was immersed in 10% phosphate-buffered formol saline for 10 days to preserve the strained condition of the skin.

Specimens for scanning electron microscopy were prepared by the method previously described in detail by Brown (1972). Frozen tissue was sectioned by sledge microtome, but the sections were discarded, as it was the exposed surface of the bulk tissue which was examined in the SEM. Each strained specimen provided four microscope specimens. Of these, three were sectioned horizontal to the epidermis to expose; (a) the superficial area of the dermis, (b) the mid-dermis, and (c) the deep dermis. The fourth specimen provided a complete vertical section of the skin.

Sectioned specimens were immersed in an aqueous solution of hyaluronidase (20,000 IU/l) for 24 h at 37 °C, with occasional agitation. Dehydration by alcohols and vacuum desiccator followed. Specimens were attached to the SEM stubs, coated with 20 nm gold–palladium, and examined in a Cambridge Stereoscan Mark 2A scanning electron microscope. Micrographs were recorded on 120 size Ilford FP4 film using a Zenza Bronica camera.

RESULTS

The load–extension records of the skin specimens demonstrated the classical three phase form as shown in Fig. 1. The initial large extension at loads less than 10 g formed phase 1, the elbow of the curve, at 10–300 g, was phase 2, and the almost linear portion at loads greater than 300 g represented phase 3. Specimens from a given sample behaved similarly over their common load range, thus allowing the structure of sequentially strained specimens to be related.

The epidermis has a two-stage response to progressive straining. Initially its undulating form is pulled out flat (Figs. 2 and 3). Further extension of the skin elongates the cells themselves (Fig. 4). The strain at which cell elongation begins can vary from near the end of phase 1 to the middle of phase 2 of the load–extension curve. When stretched, the cells of the non-keratinized layers assume ellipsoid shapes: in many cases the stratum corneum was washed off during processing and could not be studied. When strained well into phase 3 of the load–extension curve, the cellular layers are so compacted that they cannot be individually distinguished in edge view, although in sections horizontal to the surface the intercellular spaces are still distinct (Fig. 4).

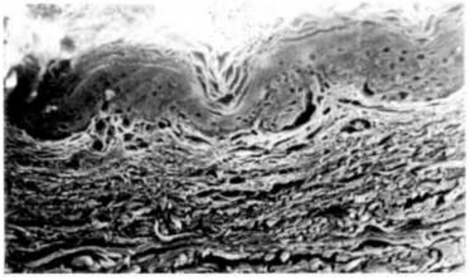

FIGURE 2. Unstressed skin. An edge view showing the undulating form of the epidermis (× 140).

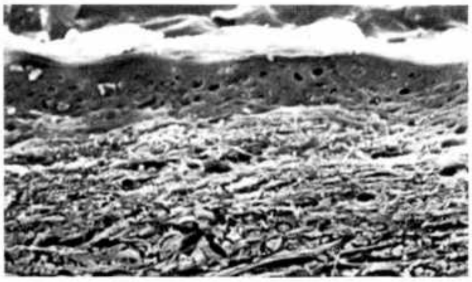

FIGURE 3. Strain level: beginning of phase 2. The epidermis is pulled into a flat layer (× 155).

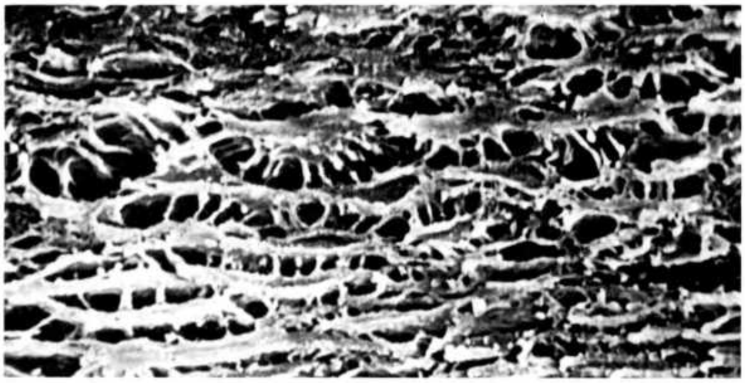

FIGURE 4. Strain level: mid phase 3. Section parallel to surface showing elongated cells of the stratum spinosum (× 2080).

The papillary layer is the layer of fine fibres immediately underlying the epidermis. The effect of strain on the papillary fibre networks is initially reorientation to the load direction, followed by compaction at higher loads. The occurrence of these changes is dependent on the behaviour of the epidermis, rather than being related to the phases of the load–extension curve. The initial deformation of the network produces loosely aligned streaks of fibres. This coincides with the epidermis being drawn out flat. As the strain is increased, the fibres become more aligned and compacted together.

Progressive straining causes the coarse fibres of the mid-dermis to reorientate towards the load axis, straighten and become aligned in parallel arrays (Figs. 5–8). These changes are related to the phases of the load–extension graph. At the end of phase 1 a few fibres are straight, but most are tortuous and

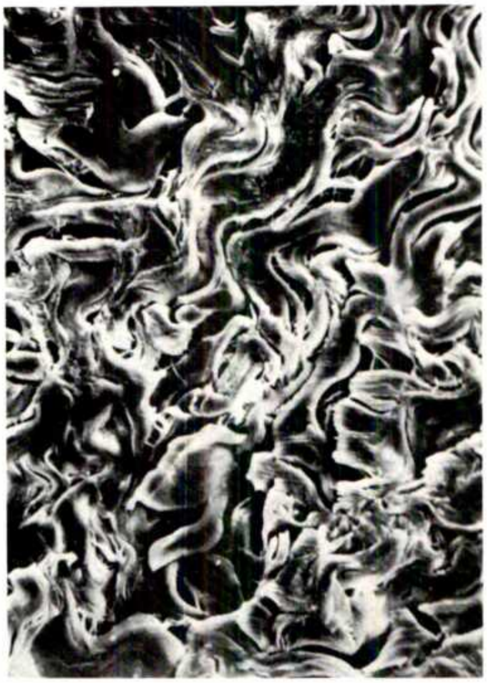

FIGURE 5. Unstressed skin. Parallel section through mid-dermis showing multidirectional system of wavy fibres (× 225).

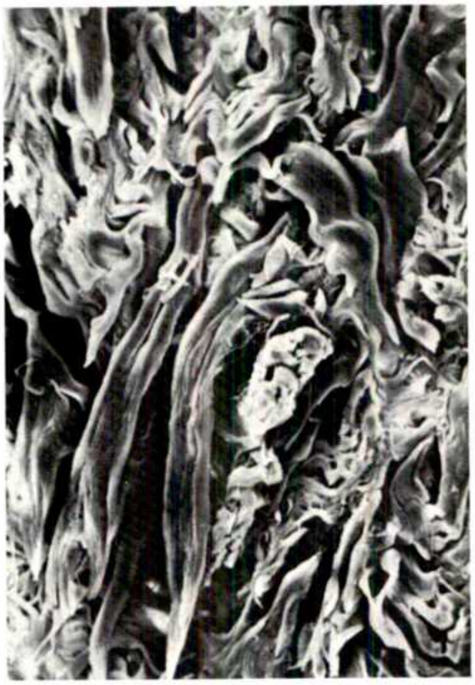

FIGURE 6. Strain level: end of phase 1. Fibres have reoriented towards load axis, and a few are nearly straight (× 225).

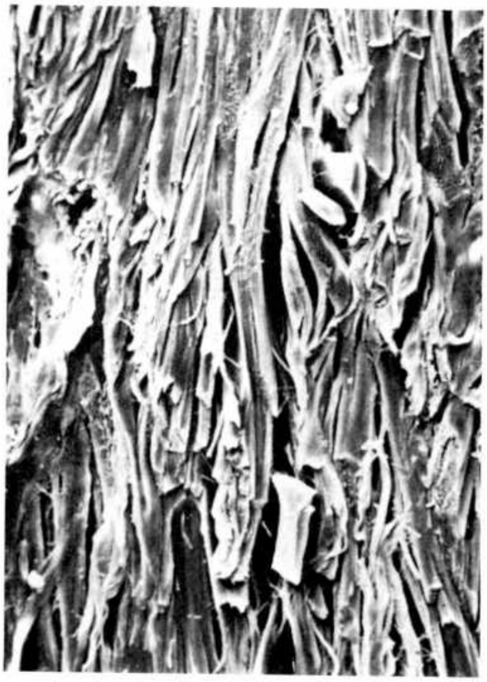

FIGURE 7. Strain level: end of phase 2. Fibres are aligned and almost straight (× 225).

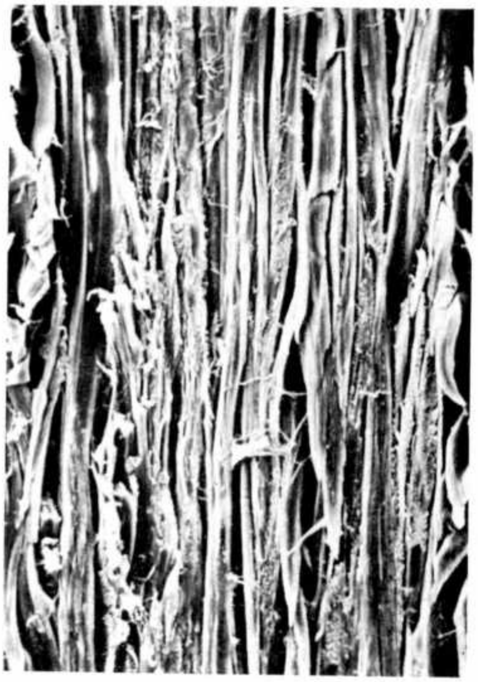

FIGURE 8. Strain level: mid-phase 3. Fibres are aligned, straight, and compacted (× 225).

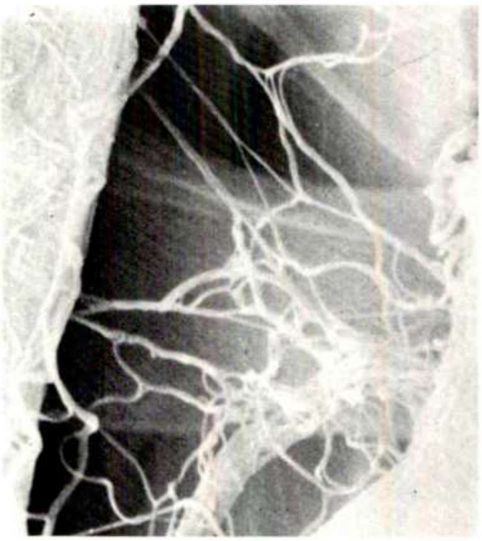

FIGURE 9. Unstressed skin. Inter-fibre fibrils are loosely arranged (× 4,800).

tend to be more oriented towards the load axis, and parallel to the surface, than in the unstressed condition (Fig. 6). During the second phase the fibres continue to reorientate and straighten, until by the end of this phase the fibres are almost straight and aligned (Fig. 7). As the skin is extended into phase 3 the fibres become compacted together (Fig. 8). In this condition a greater length of individual fibres is visible than in the tangled mass of the unstressed specimen, but no natural ends were identified. Between the fibres, the network of inter-fibre fibrils is stretched as the skin is extended (Figs. 9 and 10).

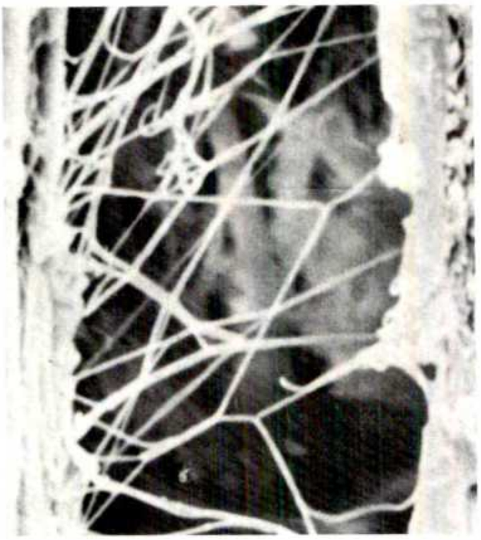

FIGURE 10. Strain level: mid-phase 2. Inter-fibre fibrils are taut (× 5000).

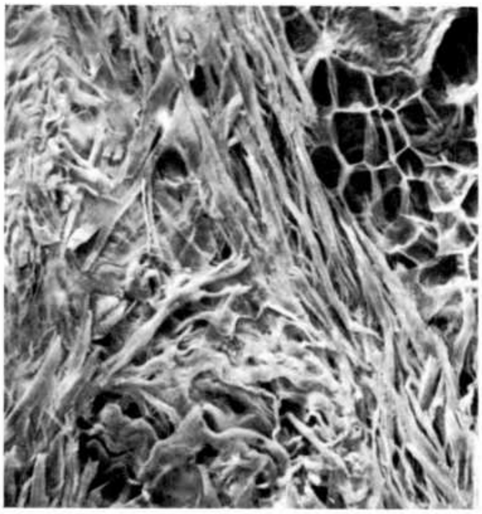

FIGURE 11. Strain level: end of phase 1. Streaks of oriented fibres lie around fat cells (×80).

Fibres in the deeper parts of the dermis behave similarly to those in the mid-dermal region, although the mid-dermal changes are slightly in advance of those in the deep zone. A feature of the deep zone is the response to straining of the groups of fat cells and the fibres surrounding them. In the first two phases of extension, the fat cells are more oriented than the general fibre network. At the end of phase 1, fibres lying close to the fat cells are oriented in the load direction in aligned streaks, while most fibres in the deep dermis are still tortuous (Fig. 11). At greater extensions, the honeycomb structure of the fat cells is elongated (Fig. 12).

The behaviour of hair follicles, glands and the fibres surrounding them during progressive straining is similar to that of fat cells and their adjacent fibres (Figs. 13 and 14). At the end of phase 1, streaks of oriented fibres lie close by the follicles and glands, while most of the dermal fibres are still tortuous.

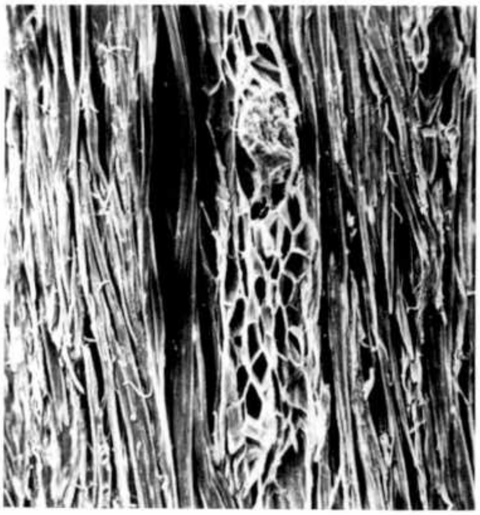

FIGURE 12. Strain level: mid-phase 3. Fat cells are elongated (×70).

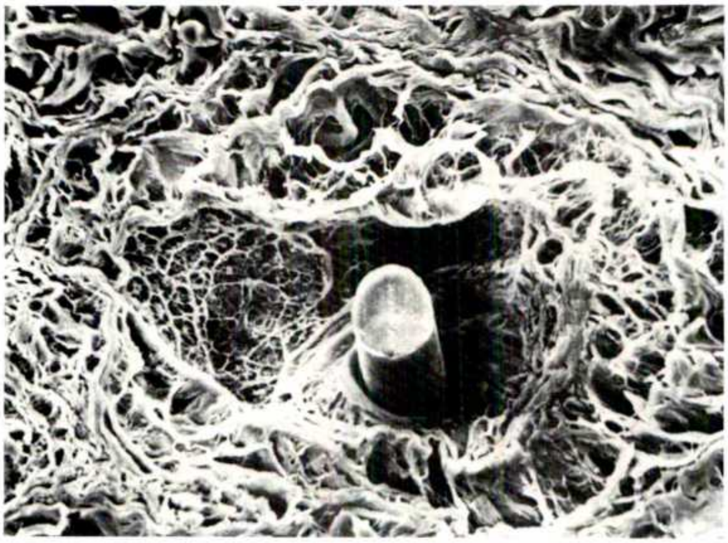

FIGURE 13. Unstressed skin. Section parallel to surface through the mid-dermis showing hair shaft, follicle, gland, and surrounding fibres (× 168).

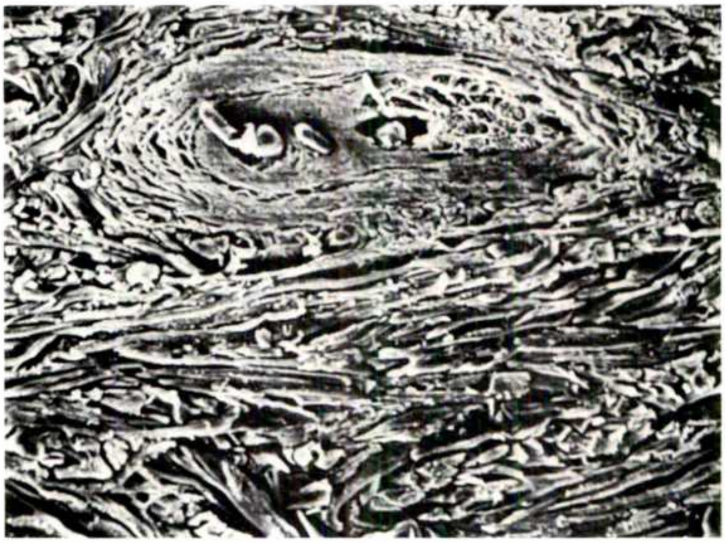

FIGURE 14. Strain level: end of phase 2. The hair follicle and gland is distorted (× 144).

Distinct elongation of follicles and glands is evident by the second half of phase 2 (Fig. 14). Distortion of these structures becomes more severe as the skin is extended. However, the glandular bodies do not collapse even at strains well into phase 3.

DISCUSSION

Strain produces a two-stage effect on the epidermis. Initially the concertina form is pulled out flat, and further extension elongates the cells. The strain at which cell elongation begins depends on the waviness of the epidermis, but it is unlikely that the strains produced by normal physiological activity significantly distort the cells. The independence of the mechanical behaviour of the epidermis from that of full-thickness skin is an indication that the epidermis does not contribute significantly to the mechanical properties of abdominal skin. More direct evidence of this has been obtained from mechanical studies of abdominal skin with the epidermis incised, to prevent it contributing mechanically (Daly, 1966). The toughest layer of the epidermis is the stratum corneum (Wildnauer, Bothwell & Douglas, 1970; Wildnauer, 1970). These studies have shown that the mechanical effect of the epidermis is negligible when it is much thinner than the dermis. However, where the epidermis is relatively thick, as on the palms and soles, it is likely to have a significant effect on the skin's mechanical properties (Harkness, 1971).

In general terms, the observations of the effect of strain on dermal fibre arrangement confirmed those described by previous workers, notably those of the principal investigators (Craik & McNeil, 1965; Gibson, Kenedi & Craik, 1965). In response to progressive straining, fibres throughout the dermis straighten, reorientate towards the load direction, and become compacted. Fibres in the mid-dermis are affected in advance of the deeper regions. However, deformation of the papillary layer is dependent on the behaviour of the epidermis rather than on lagging behind the mid-dermis, as Craik & McNeil (1965) described. In certain plastic surgical reconstructive procedures 'blanching' (a lightening of the skin colour) occurs when the skin is strained to a level corresponding to the middle of phase 2 (Gibson & Kenedi, 1967). Strains of this magnitude cause the papillary layer fibres to be well aligned. It is likely that deformation of the papillary layer fibres, and possibly of the capillaries themselves, cause these vessels to be occluded, resulting in necrosis of the area if allowed to persist.

Extensibility of the skin is limited to that allowed by deformation of the fibre network, as little extension is possible once the fibres are aligned. The deformability of the fibre network is determined by the configuration of the wickerwork in the unstrained condition. Thus, in cases where hyperextensibility is due to abnormally high strains in phase 1 of the load–strain curve (e.g. the Ehlers-Danlos syndrome; Grahame & Beighton, 1969), a network which allows great fibre mobility is likely to exist.

The stiffness of the skin during the third phase of extension (indicated by the slope of the graph) and the tensile strength of the skin are determined by the properties of the aligned fibre system. Since more than 90% by weight of the dermal fibres is collagenous (Weinstein and Boucek, 1960), the *properties* of the collagen fibres, or the *strength* of the interconnections between them, whichever is least, will determine the *performance* of the skin in the high load range. The properties of collagen may be susceptible to change through modification of the ultrastructure, e.g. by increased cross-linking. The means of load transference between fibres remains an enigma, despite high magnification examination of strained specimens. The interfibre fibrils are clearly unable to transmit high loads. No natural ends to the fibres were identified, and so it was not possible to ascertain whether the networks are composed of a continuous interconnected system, as Dempsey (1968) has observed in animal skins, or if the fibres terminate by division into the smallest subunits, like the branches of a tree. Continuous networks have obvious mechanical strength, but if the fibres are separate and tangled, the tensile strength of the skin will depend on friction between fibres, in the same way as twine. In this case the properties of the ground substance adhering to the surface of the fibres could affect tensile strength by the nature of its fibre lubrication properties, which may be alterable by physiological or pathological conditions.

The main function of elastin fibres in skin is to act as a return spring, by making the skin contract on the removal of an applied load (Daly, 1966). Elastin fibres are unlikely to contribute significantly to the load deformation behaviour of normal skin, due to its long range extensibility and the comparatively small quantity present (4% of the fat-free dry weight; Weinstein & Boucek, 1960). The effect of strain on the elastin network could not be determined in this study, since it is not possible to 'label' tissue components for identification when using the SEM in the emissive mode.

In the immediate vicinity of hair follicles, glands, and aggregations of fat cells, the fibres become taut at lower strains than in the general networks. These non-fibrous structures are likely to limit the mobility of the fibres. Thus, variation in the mechanical properties of skin from different sites could be due, in some cases, to variation in the density of hair follicles, glands or fat cells. The scalp, for example, has low extensibility and a high density of hair follicles.

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The aerobic cutaneous microflora of diabetic subjects

DOROTHY A. SOMERVILLE* AND M. LANCASTER-SMITH

Department of Bacteriology, St John's Hospital for Diseases of the Skin, London E9 6BX,
and the Division of Medicine, The London Hospital, London, E.1

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SUMMARY

The microbial skin flora in a group of ninety-eight adults with diabetes mellitus requiring treatment with insulin is described. As in healthy adults with a similar age range, the dominant members of the flora are the micrococci and diphtheroids. *Sarcina* species and *Micrococcus* Baird-Parker Group 7 were more common than in the general population, and there was a higher incidence of erythrasma and increased carriage of fluorescent diphtheroids.

Diabetes mellitus has long been recognized as a predisposing factor in superficial and systemic fungal infections, particularly candidiasis and mucormycosis (Baker, 1960; Landau & Newcomer, 1962), while infective cutaneous complications, such as vulvitis, balanitis, and pyoderma arise frequently (Younger, 1965). In diabetes, there is an increased glucose store in the dermis (Rothman, 1954; Fusaro & Goetz, 1971), and the level of cutaneous free-glucose is higher than in the general population, particularly when blood glucose levels are over 180 mg/100 ml, for epidermal glucose levels are related to blood glucose levels (Shragger, 1962; Zaias, Shragger & Cushner, 1963).

It has often been suggested that diabetic subjects carry more pathogenic bacteria on the skin than healthy individuals, leading to the reputedly high rate of furunculosis and foot infections (Marples, 1965; Smith, O'Connor & Willis, 1966). Sufficient numbers of uncontrolled diabetics were not available for study, and it was, therefore, decided to study the microbial skin flora of well-controlled, insulin-requiring diabetics; patients attending the London Hospital Diabetic Clinic being chosen. Ninety-eight patients, fifty-four males and forty-four females, were included; only those under 65 years were studied, as the flora of elderly people differs from that of younger adults (Somerville, 1969).

MATERIALS AND METHODS

It is difficult to assess the degree of day-to-day control of blood glucose levels achieved by diabetic outpatients with any certainty, and there is no clearly definable measure of severity in this disease. It was, therefore, not possible meaningfully to designate patients as having mild or severe disease. At the time of the examination, a blood glucose concentration was estimated and note was made of the patient's age, duration of disease, insulin requirements, presence of obvious skin infections and of any complications related to the diabetes, such as neuropathy, retinopathy, peripheral vascular disease, and pruritus vulvae (Table 1). Correlation of these definable parameters with the skin microflora was attempted.

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* Present address: Department of Bacteriology, Royal Infirmary, Glasgow C4 0SF.

The toe-webs, axillae and groins of all subjects were examined for erythrasma using a Wood's UV light, and note was made of any fluorescence, scaling or maceration of the area. Samples were taken from all individuals using sterile cotton-wool swabs moistened in nutrient broth, and the specimens were treated as described by Somerville (1969). Areas selected for sampling were the nose, gums, chest, periumbilical region, groin, thigh, shin, scapular area, lumbar region of the back, forearm, 4th toe-web, axilla, cheek, forehead and ear. Any areas showing fluorescence were also swabbed. Swabs from these fluorescent areas, and from the axillae, groins and toe-webs were also inoculated onto Tissue Culture Medium No. 199 (Wellcome) for the isolation of fluorescent diphtheroids, as described by Somerville (1970).

TABLE 1. Details of the ninety-eight diabetic subjects included in this study

	Males	Females
Number	54	44
Age		
<45 years	23	17
≥45 years	31	27
Insulin requirements		
<50 units	19	29
≥50 units	35	15
Blood glucose levels (mg/100 ml)		
<120	17	14
120–179	15	13
≥180	22	17
Diabetic complications		
Retinopathy	7	3
Neuropathy	5	5
Nephropathy	2	1
Cataract/glaucoma	5	1
Peripheral vascular disease	3	1
Pruritus vulvae	—	2
Foot infection	2	—
Others	2	5
Total with one or more complications	19	12

All micro-organisms isolated were recorded, and a semi-quantitative estimation of their numbers made (± = <5 colonies; + = 5–20 colonies; ++ = >20 colonies). The organisms were identified and grouped into the following groups; the Micrococcaceae, including *Staphylococcus aureus*; streptococci; diphtheroids; other Gram positive bacilli; Gram negative bacilli, including enteric organisms and those belonging to the tribe Mimeae. Micrococci were grouped by the scheme of Baird-Parker (1965), retaining the genus *Sarcina* to describe the aerobic, packet-forming Gram positive cocci with yellow colonies; the fluorescent diphtheroids were grouped according to Somerville (1970). Phage typing of the *Staph. aureus* isolates was carried out in our own laboratories, using the standard phage preparations supplied by the Public Health Laboratory Service. Yeasts isolated were identified using the criteria of Lodder (1970).

RESULTS

It is difficult to determine whether an organism found on the skin is actually living and multiplying on the skin or whether it is a transient contaminant. For the purpose of this paper an arbitrary level has been chosen, and the term 'colonization' is used when more than 5 colonies of the organism were isolated from a site by the above technique.

Strains of coagulase negative staphylococci and micrococci were found in large numbers on the skin of all subjects examined. The most commonly found were those in Baird-Parker Groups S2, M1, M2, M5, and M7, and *Sarcina* species (Table 2). *Staph. aureus* was isolated from 57% of patients, and in large numbers from 48%. Heavy nasal colonization occurred in 29%; 13% were colonized by the same strain on the skin and in the nose, while skin colonization without nasal carriage occurred in 20%. No single phage type predominated.

TABLE 2. Percentage carriage of Micrococcaceae at specified sites in ninety-eight adult diabetics

Site	S1 (*Staph. aureus*)	S2	S3	S6	M1	M2	M3	M5	M7	*Sarcina*
Nose	32	20	3	3	16	34	6	12	9	12
Chest	15	20	2	1	27	40	8	21	17	57
Periumbilicus	11	15	1	1	33	41	7	26	16	44
Groin	7	16	5	5	33	43	10	20	9	26
Thigh	10	6	6	5	36	34	15	24	15	43
Shin	11	7	2	2	33	27	7	22	28	52
Scapula	9	9	3	5	29	34	5	22	23	57
Lumbar back	6	10	2	1	31	38	10	23	21	43
Forearm	10	5	5	5	22	40	12	29	27	54
Toe-web	5	13	17	3	26	22	7	44	17	14
Axilla	4	21	5	2	28	41	8	15	3	37
Cheek	23	36	8	3	34	44	10	21	13	42
Forehead	23	33	9	1	34	37	7	21	15	19
Ear	20	24	5	1	29	37	13	20	33	22

Diphtheroids were also very commonly isolated from the skin, the majority of sites being heavily colonized (Table 3). Differentiation into lipophilic and non-lipophilic diphtheroids was not attempted. Erythrasma (which for the purpose of this paper is defined as a scaling area of skin fluorescing coral-red under the Wood's light) occurred in 47% of the diabetics examined, and was more common in the males than the females, and in those over 44 years (Table 4). Fluorescent diphtheroids could be isolated from apparently healthy areas in 61% of the diabetics, and carriage in the groins was most common (Table 5). Colonization was greatest in those with a blood glucose level higher than 180 mg/100 ml.

Organisms other than micrococci and diphtheroids were not major members of the skin flora; a small proportion of all sites carried Gram positive bacilli other than diphtheroids, streptococci, and Gram negative bacilli (Table 3).

Candida albicans was found on the gums of 19% of the diabetics and on the skin of 13%. Skin

TABLE 3. Percentage colonization† of the skin by various organisms at specific sites in ninety-eight adult diabetics

Site	Diphtheroids	Other Gram positive bacilli*	Enteric organisms	Mimeae	Streptococci
Nose	87	3	1	—	6
Chest	65	14	7	6	6
Periumbilicus	83	11	2	1	3
Groin	88	8	7	—	2
Thigh	74	15	2	3	5
Shin	59	16	3	3	2
Scapula	50	15	2	3	1
Lumbar back	64	12	—	—	7
Forearm	63	15	5	4	6
Toe-webs	91	4	2	—	8
Axilla	68	8	6	2	1
Cheek	51	10	5	3	6
Forehead	47	10	2	3	3
Ear	44	7	—	—	4

* Most of these were seen to be spore-bearers

† More than 5 colonies of each organism isolated on primary culture from colonized site.

sites most commonly involved were the groins and toe-webs; only 4% carried *C. albicans* both in the mouth and on the skin. Yeasts other than *C. albicans* were found on the gums of 14% and on the skin of 31% of the subjects, and were more common on the skin of women. The most frequently isolated species were *C. parapsilosis* and *Torulopsis glabrata*.

There was no significant difference in the composition of the microbial flora between those requiring less than 50 units of insulin per day and those needing more. Duration of disease did not appear to affect the cutaneous flora, nor did those patients with medical complications of their disease

TABLE 4. Percentage incidence of erythrasma in ninety-eight diabetics

	No. examined	Axillae	Groins	Toe-webs	Any site
Males	54	6	9	54	56
Females	44	2	7	32	36
Total	98	4	8	44	47

TABLE 5. Percentage carriage rate of fluorescent diphtheroids in non-fluorescent skin sites

	No. examined	Axillae	Groins	Toe-webs
Males	54	26	37	11
Females	44	16	48	20
Total	98	21	41	15

show any significant differences from those without diabetic complications, though skin carriage of *C. albicans* was higher, and erythrasma apparently more common. Only ten patients had a high insulin requirement, a high blood glucose level, and complications of the disease; these patients, as a group, did not show any significant differences in skin flora from the other diabetics examined.

DISCUSSION

Diabetic subjects have a similar type of normal flora to comparable healthy young adults; micrococci and diphtheroids predominate, and there is a minor resident flora of streptococci, Gram negative organisms and yeasts in some sites. Carriage of *Staph. aureus* on the skin and in the nose was not significantly higher in these subjects than in healthy populations studied by several workers (Williams, 1963).

However, colonization with *Sarcina* species and *Micrococcus* Group 7 occurred more commonly than in young adults studied by Noble (1969) and Somerville (1969). Organisms belonging to both these groups do not attack glucose during growth, and it is strange that their incidence should be high on the skin of subjects who are expected to have a higher than normal level of cutaneous free-glucose. Streptococci and Gram negative bacilli were less common than in the healthy population (Somerville, 1969; Somerville & Noble, 1970). Unexpectedly, the incidence of yeasts was not significantly higher in this series than in healthy adults.

Erythrasma occurred more frequently in the diabetic group than in young adults, but there were no extensive infections. Even mild erythrasma and the carriage of fluorescent diphtheroids on unaffected sites is more common in those with diabetes mellitus than in the general population, reinforcing Montes' suggestion (Montes *et al.*, 1969) that diabetics may have an increased susceptibility to this infection.

With the exception of erythrasma and its causative organisms, well-controlled diabetics have a similar skin microflora to that of non-diabetics of the same age. Even patients with high blood glucose levels, high insulin requirements and medical complications had normal skin flora.

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Mites in the personal environment and their role in skin disorders*

MARK HEWITT, G.I.BARROW, D.C.MILLER, FRANK TURK
AND STELLA TURK

Department of Dermatology, Cornwall Clinical Area; Public Health Laboratory,
Royal Cornwall Hospital (City), Truro; and the University of Exeter Department
of Extramural Studies in Cornwall

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SUMMARY

An investigation into the incidence and distribution of 'ectoparasites' in the close personal environment of patients with chronic unexplained dermatoses is described. Acari were found in 356 samples from 124 of 253 (50%) patients investigated. The house dust mite *Dermatophagoides pteronyssinus* and related species accounted for 157 (44%) of the 'parasites' identified. The clinical and laboratory parasite recovery techniques are described and their limitations considered. The possible significance of the results is discussed and the need for further work emphasized.

Mites, which belong to the order *Acari*, differ from insects, such as lice, by fusion of the head and thorax, by the absence of all primary segmentation and by possession of four pairs of legs. The complex life cycle of mites includes egg, larval, nymph and adult stages, the latter being barely visible to the naked eye. Many have become adapted to life as facultative or obligatory parasites, having remarkable tenacity and powers of reproduction. Although implicated in both human and animal dermatoses (Zumpt & Graf, 1950; Baker *et al.*, 1956) acari other than scabies mites have perhaps received insufficient attention from dermatologists, although dermatoses from infested copra (Castellani & Hirst, 1912), straw (Booth & Jones, 1954) and stored foods (Hughes, 1961) have long been known. More recently, the importance of mites in dust, some of which are identical with those found in stored grain and food, has been studied (Fain, 1966, 1967). In particular, members of the genus *Dermatophagoides* have now assumed a significant role in the aetiology of asthma and allergic rhinitis (Spieksma, 1967; Bronswijk, 1971).

Parasitic infestation of pet animals has also been associated with human skin lesions; forage mites and their eggs being second only to fleas in importance (Hewitt, Walton & Waterhouse, 1971). In further work, mites were also found in some cases of unexplained dermatoses in which pet animals were not concerned. The present paper describes four illustrative cases, as well as subsequent investigations into the role of mites in the personal environment of other patients.

MATERIAL AND METHODS

Patients

During a period of 18 months a total of 356 specimens were obtained from 253 patients with various unexplained dermatoses who were investigated for the presence of mites in their personal environment.

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Shakings from unlaundered clothes in contact with the skin were obtained from 206 of these 253 patients. Vests, pants, knickers, bras, shirts or pullovers were turned inside out and well shaken into large plastic bags. The air was expelled from the bags, which were then sealed with Sellotape and labelled. Samples of floor dust from home or work associated with twenty-five of the patients were obtained by brushing and collecting on sterile sheets of brown paper, which were carefully folded and sealed. Upholstery brushings from chairs and settees associated with eleven patients were similarly obtained, and bed linen and sheets from thirty-six of the patients were shaken into large plastic bags. Skin brushings from seventy-eight of the patients were taken by means of sterile nylon CSSD brushes into Petri dishes. All these specimens were submitted for laboratory examination.

Control patients

As controls, clothes-shakings similarly obtained were examined from two groups of patients with no history of skin disease. These included: (a) sixty-six children admitted to hospital for surgical operations; and (b) fifty-one adults attending either a general practitioner's surgery or hospital outpatient clinics for minor surgical procedures.

Laboratory examination for parasites

Centrifugation method. Clothes, chairs, or bedding were brushed or shaken into large heavy duty plastic bags. In the laboratory, about 20 ml of solution A (Gisin, 1955), which relaxes the legs and appendages of parasites and then 'fixes' them, was poured in and the bag thoroughly shaken. This fluid was then poured into a wide necked jar. The process was repeated twice, and the jar of fluid left at room-temperature overnight. The surface of the fluid was 'skimmed' with glass microscope slides smeared with lanolin, and the fluid then transferred to universal containers for low-speed centrifugation at 200 rev/min for 3 min. The fluid was carefully removed with a pasteur pipette to within 1·25 cm of the bottom of the container. The remainder of the fluid was transferred to further lanolin slides with a wide-bore pipette. All the lanolin slides were allowed to dry in air at room temperature, and then examined with a plate microscope. Any parasites or suspect material were removed with a fine wire and placed in a drop of examination solution B (Gisin, 1955) on a microscope slide for clearing and subsequent identification. After confirmation by low-power microscopy, a cover slip was placed on the slide and the position of the mite marked with waterproof ink.

Flotation method. Brushings from animals, floors and other dirty materials usually require a different technique because of the large amount of amorphous debris which may mask any mites. The material was split, one part being examined by the centrifugation method already described. The other part was examined by a modification of the method described by Cornere (1971). It was placed in a large (6 in)

TABLE 1. Formulae of solutions A and B

Fixing solution	(A)	Examination solution	(B)
90–95% alcohol	750 ml	90% lactic acid	100 ml
Solvent ether	250 ml	Glacial acetic acid	4 ml
Glacial acetic acid	30 ml	Gelatin	4 g
40% formalin	3 ml		

glass Petri dish, covered with 95% lactic acid[*] and incubated overnight at 37°C. This allows particulate matter to settle at the bottom, whereas mites and light debris rise to the surface, which is then scanned with a stereoscopic low-power microscope. Any parasite or suspect material is picked with a straight wire into a drop of examination solution B on a microscope slide and a cover slip added as before.

Identification. After initial confirmation and preliminary identification, all slides with mites, whether complete or not, were referred for precise species classification according to recognized acarological procedures.

When identified, only one member of each species was attributed to any specimen, so that it is tabulated once per specimen. Any species recovered from more than one kind of specimen associated with one patient was listed separately under the appropriate heading. However, more than one sample was taken from several patients.

RESULTS

Of the total of 253 patients investigated, acari were found in the close environment of 124 of them. Of these 124 patients, clothes shakings from ninety-one yielded 170 'ectoparasites,' of which the house dust mite *D. pteronyssinus*, the related species *D. farinae* and *Euroglyphus maynei* were by far the most common (Table 2). In addition, *Glycyphagus* species and *Acarus siro*, both known to cause dermatoses, were present in clothes shakings from fifteen and ten patients respectively. On the other hand, *Gohieria fusca*, which has not previously been described in association with skin lesions, was found in fourteen instances from the environment of eight patients. As might be expected, bedding and upholstery yielded mainly house dust mites. However, Cheyletid species, which are known to be

TABLE 2. Acarine species found in the personal environment of 124 patients[†]

Acari	Bedding	Clothing	Floor dusts	Skin scrapings	Upholstery	Total
Acarus siro L.	2	10	3	1	0	16
Tyrophagus spp.	1	6	4	0	0	11
Glycyphagus spp.	3	15	6	0	1	25
Gohieria fusca (Oudemans)	2	8	0	1	3	14
Dermatophagoides farinae (Hughes)	3	2	0	0	0	5
D. pteronyssinus (Trouessart)	28	110	4	4	6	152
Euroglyphus maynei (Cooreman)	2	8	0	0	0	10
Tarsonemids (*Tarsonemus*, *Tarsonemoides* and *Pygmephorus* spp.)	3	3	0	0	0	6
Cheyletids (*Cheyletus*, *Cheyletiella* and *Cheyletophanes* spp.)	7	8	1	0	1	17
Total	51	170	18	6	11	256

[†] Only the species commonly found in the course of this work are shown. Altogether thirty-eight species were identified, but many occurred only once.

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[*] Concentrated lactic acid is corrosive to skin and care must be taken.

predators of other mites, were recovered in seventeen instances from the environment of seventeen patients, mostly from bedding and clothes. Only those species found most often during the course of this work are shown in Table 2, but altogether thirty-eight different species of epizoics were identified, although some of them occurred only once. In all, mites were found in nearly half of the patients, but their presence was not related to the severity or extent of the skin lesions, nor were they significantly associated with dirty clothes or unkempt persons.

The results obtained from clothes-shakings from the control groups are compared with those from patients in Table 3. In the controls, the house dust mite, as expected, was the commonest finding; it occurred in about 20% of the children and nearly one-third of the adults. Altogether, *Dermatophagoides* species were recovered from nineteen of 117 control persons (16%) compared with ninety-one of 206 patients with skin lesions (44%). In the control groups, the only other mites—*Tyrophagus* species and *G. fusca*—were found in eight instances from the clothes of eight patients.

TABLE 3. Species of mites from clothes-shakings from a total of 206 patients compared with those from 117 control persons

Acari	Samples from patients	Samples from controls	Total
Acarus siro L.	10	0	10
Tyrophagus spp.	6	4	10
Glycyphagus spp.	15	0	15
Gohieria fusca	8	4	12
Dermatophagoides farinae	2	0	2
D. pteronyssinus	110	19	129
Euroglyphus maynei	8	0	8
Tarsonemids (*Tarsonemus*, *Tarsonemoides* and *Pygmephorus*)	3	0	3
Cheyletids (*Cheyletus*, *Cheyletiella* and *Cheyletophanes*)	8	0	8
Total no. of mites identified	170	27	197

Some of the more common acari found during this investigation are illustrated in Fig. 1. For comparison, a male and a gravid female human scabies mite are also shown.

During these investigations, four patients have been selected from many in whom the history and clinical picture seemed to be significantly associated with mites in their personal environment. These illustrative cases are accordingly described in detail.

CASE REPORTS

Case 1

An 18-year-old youth who worked in his parents' pet food and ironmongery shop was admitted to hospital with very severe atopic dermatitis. When in hospital, he suffered intense irritation of the legs and feet every time the ward floor was cleaned, which suggested allergy to dust. In view of this, dust and sweepings from the shop floor were taken. These contained large numbers of mites, including *Acarus siro*, *Glycyphagus destructor*, *Tyrophagus fungivorus* and *Dermatophagoides* species. This dust was subsequently used in patch tests: (a) by itself; (b) after autoclaving; and (c) as an

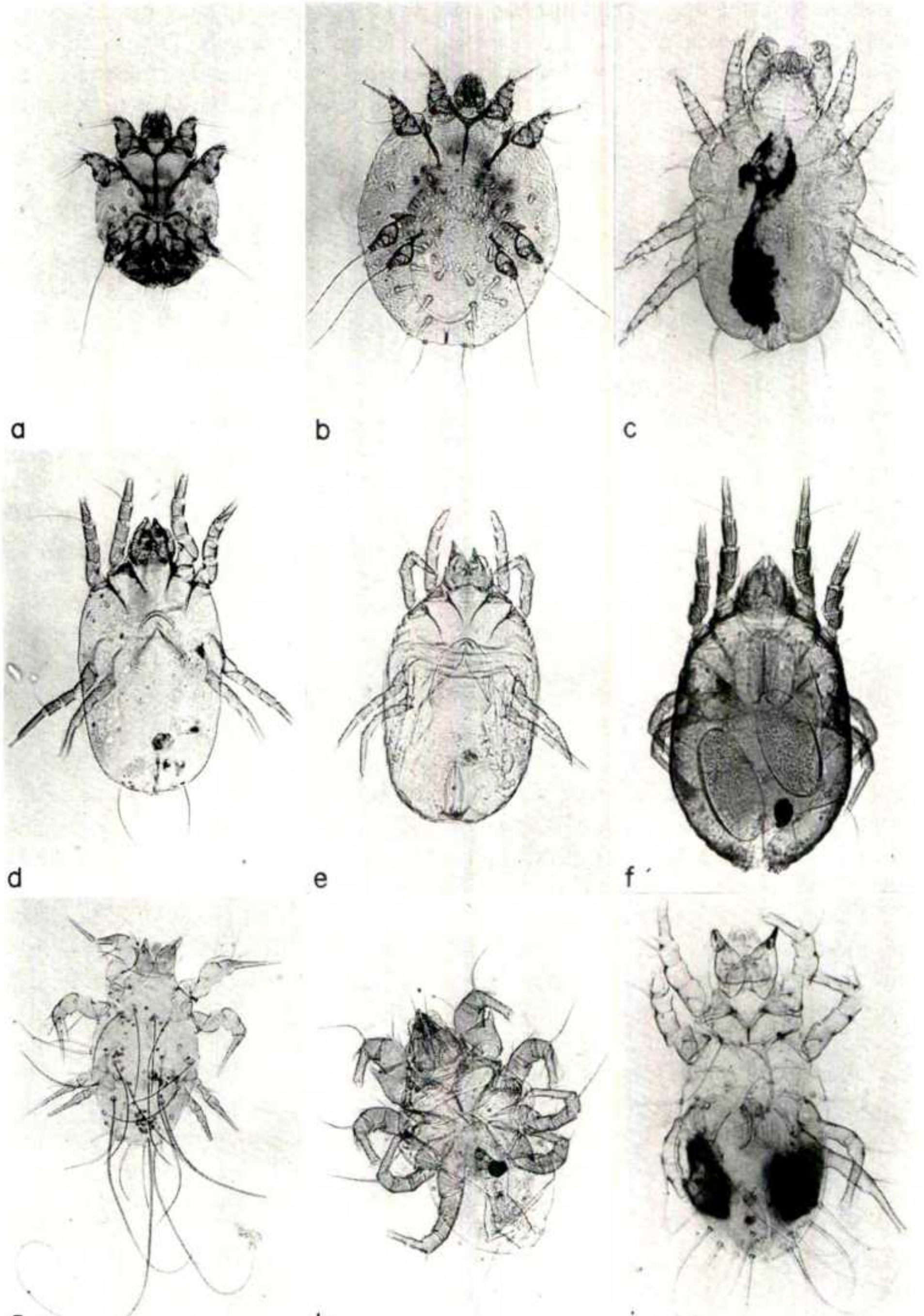

FIGURE 1. Mites found during this work compared with *Sarcoptes scabei*. (All × 200). (a), *Sarcoptes scabei* (male); (b), *Sarcoptes scabei* (gravid female); (c), *Cheyletiella yasguri*; (d), *Dermataphagoides pteronyssinus* (female); (e), *Euroglyphus maynei* (female); (f), *Gohieria fusca* (female); (g), *Glycyphagus ornatus*; (h), *Acarus siro* (male); (i), *Tyrophagus palmarum* (female).

alcoholic extract (Champion, 1971). All were positive, the autoclaved dust giving the strongest reaction; 0·5% phenol in saline, used as a control, produced no reaction. The patient's condition gradually improved with steroids by mouth. On his return home, anti-dust measures, including covering the floor with hardboard and his mattress with plastic sheeting were adopted in his bedroom, and the shopfloor was treated with a Lindane insecticide. He later left the shop, took an open-air job, discontinued steroids, and his condition has since remained satisfactory.

Case 2

A married woman aged 25 years with an atopic history, living in an old damp cottage, presented with extensive chronic eczema of 3 years' duration affecting the arms, face and trunk (Plate 1). Although initial patch tests had revealed sensitivity to nickel, she made little progress despite advice and steroid therapy. When repeated 2 years later, patch tests were negative. Subsequent examination of clothes, sheet shakings, and chair brushings all showed the presence of mites, including *D. pteronyssinus* and *D. farinae*, *Cheyletus eruditus* and *Euzetes seminulum*. Patch tests were then done with the floor dust from case 1. There was a strong local reaction to the autoclaved antigen (Plate 2), associated with a severe general exacerbation of her eczema necessitating therapy with oral steroids, thus suggesting that 'ectoparasites' or their products were responsible. Skin tests to both house dust and live house dust mite antigens were strongly positive (Plate 3). Improvement in her condition commenced on moving to a new bungalow with modern furnishings. She began to relapse about 9 months later, however, when *D. pteronyssinus* was again identified in further clothes-shakings.

Case 3

A boy aged 7 years had been in hospital on three occasions during a period of three years with severe patchy eczema of the buttocks and thighs (Plate 4). On each occasion, the rash cleared rapidly after his admission to hospital, but recurred when he returned home. In the first instance, brushings from the family dog were examined and the presence of mites and eggs confirmed. The dog was treated with DDT and then relegated outside to the garage. This coincided with a marked improvement of the eczema. However, it subsequently recurred and the home environment was then investigated for ectoparasites. Sheet and mattress brushings as well as a sample of hair padding from his interior sprung mattress yielded numerous mites, including *D. pteronyssinus*, *Glycyphagus privatus*, *Acarus siro* and Tarsonemid species. House dust and house dust mite antigens gave positive reactions in skin tests but the response to dog hair was negative. Patch tests to live and autoclaved mites from case 1 were positive, and an eczematous reaction was present at the site of the autoclaved antigen 1 week later. In view of these findings, the hair mattress was replaced with one of foam rubber, and this again coincided with rapid clearing of his skin lesions. It is of interest that the hair mattress was kept indoors for some time before disposal, and minor eczematous eruptions occurred after he played near the mattress. When the hair mattress was destroyed, he remained symptom-free for nearly 1 year, although the eczema has since recurred.

Case 4

A man aged 45 years presented with a 14-year history of a recurrent, discrete papular eruption accompanied by severe irritation of the head, neck and shoulders similar to that described by Traver (1951). Scrapings and brushings of the skin from the affected area yielded *Chortoglyphus arcuatus*, and clothes shakings contained *D. pteronyssinus*, *Gohieria fusca* and *Glycyphagus privatus*. Skin tests to house dust and house dust mite antigens were positive. Irritation and slight erythema occurred after 48 h at the site of the patch tests to the autoclaved mites from case 1. After prolonged and varied treatment, his skin lesions cleared rapidly with the application of Neomedrone lotion.

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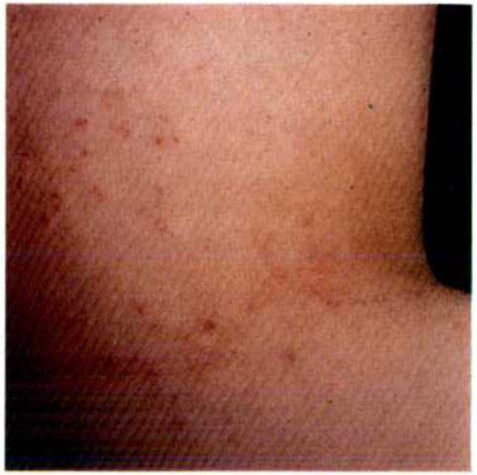

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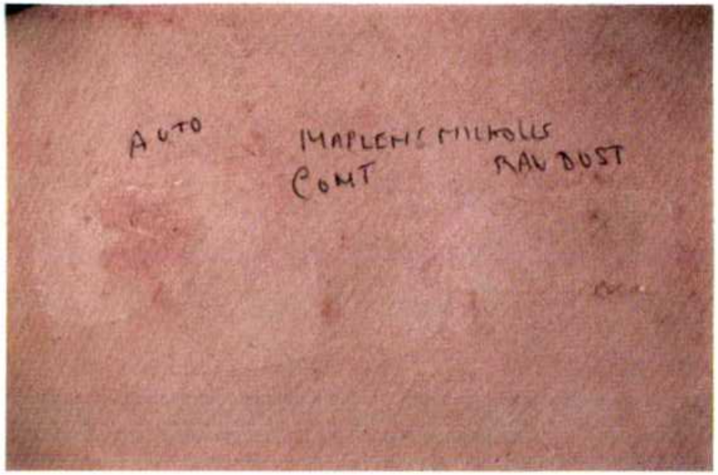

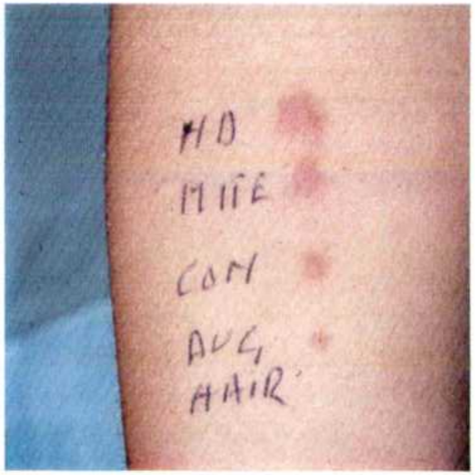

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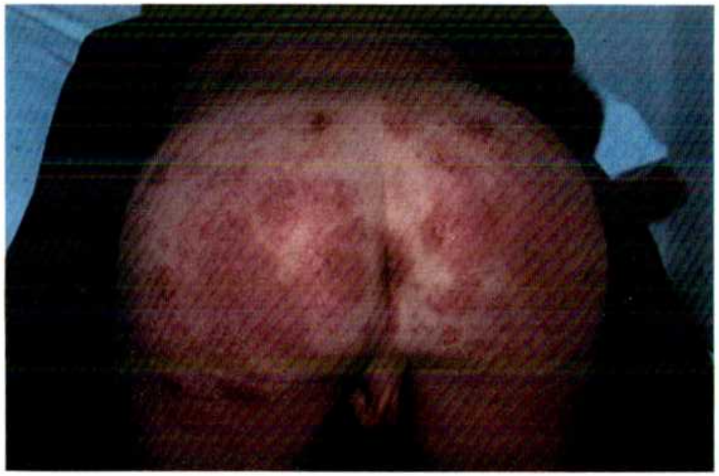

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PLATE 1. Right arm of case 2 showing typical eczematous eruption.

PLATE 2. Patch tests of case 2 showing the strong reaction to the autoclaved floor dust antigen from case 1.

PLATE 3. Prick skin tests on case 2 showing the positive reaction to house dust and house dust mite antigens.

PLATE 4. Eczematous rash on buttocks of case 3.

DISCUSSION

The importance of the house dust mite, *D. pteronyssinus*, and other *Acari* in the aetiology of allergic asthma is now well known (Voorhorst, Spieksma & Varekamp, 1969). The presence of other ectoparasites associated with pet animals has also been described in patients with skin lesions (Hewitt *et al.*, 1971). Although the presence of *Dermatophagoides* species in skin scrapings from a few patients with non-specific dermatoses was described by Sasa (1950) and by Dubinin, Guselnikova & Raznatovsky (1956), the role of parasites in the personal environment of patients with skin disorders, whether or not associated with animals, has not previously been systematically investigated. A vast and scattered literature on *Acari*, but mostly foreign, has been extensively reviewed by Oudemans (1926, 1927, 1936–37); the few synoptic papers available have mainly been published in non-medical journals by acarologists (Vitzthum, 1940–42). The present study stemmed from the apparently significant role of ectoparasites in the aetiology of the severe, recurrent eczema in case 1. In the three other illustrative cases described, 'ectoparasites' were almost certainly important as contributory factors in their skin disorders.

When considered collectively, mites were in general found more readily and more often in the personal environment of patients rather than from the patients themselves. This accords with their known mode of life on skin and dandruff shed into the environment. Dust, bedding, and clothes, as well as man or animal may all provide a suitable environment in which many *Acari* can thrive, although ecological relationships between the different habitats may be complex and variable. The incidence of *Acari* from different environmental samples is shown in Table 4. In some patients a mixed population of acarine species was present, and it is possible that this may have influenced the dermatoses. 'Parasites' were apparently present more often in patients with disseminated neurodermatitis than in other dermatoses. *Sarcoptes scabei* was, however, identified in clothes shakings from three patients, in one of whom the clinical diagnosis was not obvious. However, in dermatological conditions, parasites have not previously been sought as a routine laboratory examination, and, except in particular patients, the significance of our findings is uncertain. Their validity depends on the thoroughness and care with which specimens are collected as well as the efficiency of laboratory recovery techniques. It also depends on the patients themselves, as the presence of parasites may also be related to the examination of workday rather than freshly laundered clothes. Children, for example, are usually well-washed and brought to hospital in clean clothes for admission from the waiting list, or if out-patient examination is likely to be extensive. On the other hand, emergency admissions and

TABLE 4. Comparative analysis of the results from the different environmental samples

Samples	Positive for Acari	Negative for Acari	Total	% positive
Dust	24	1	25	96
Bedding and upholstery	27	9	36	75
Skin brushings	8	70	78	10
Clothes				
Patients	91	115	206	44
Controls	27	90	117	23
Total	177	285	462	38

patients with minor complaints are more likely to be wearing workday clothes. These probably better reflect the home or working environment, where the majority of mites live and breed. Despite this, many persons who are equally exposed to an infested environment remain apparently unaffected. Although humidity and warmth are important, fitted carpets and central heating undoubtedly affect the growth and distribution of mites. Indeed, it is of interest that the illustrative cases described in the present paper confirmed that reactions are often greater to dead mites and their products than to live acari (Rokstad, 1943; Anderson & Fishman, 1948).

Most persons live in peaceful co-existence with a variety of 'ectoparasites', but, given suitable conditions, these may, like commensal bacteria, become opportunist pathogens which if not treated may perpetuate some skin lesions. On the other hand, the presence of skin lesions may provide an environment suitable for the survival and growth of certain species of parasites, including those of animal origin. Although the aetiological significance of the present results are uncertain, they do indicate the need for further work on the possible role of mites in chronic or allergic skin disorders.

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Angioid streaks and systemic disease

JOAN F.HOGAN AND CHARLES L.HEATON

Department of Dermatology, University of Pennsylvania School of Medicine, 3400 Spruce Street,
Philadelphia, and Department of Dermatology, Philadelphia General Hospital, 34th and Civic
Center Boulevard, Philadelphia, Pennsylvania 19104, U.S.A.

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SUMMARY

A review of angioid streaks is presented. Their frequency of occurrence and relation to certain disorders are discussed. They are most commonly seen in pseudoxanthoma elasticum, Paget's disease of bone and sickle cell anaemia. The basic defects of these diseases are examined. It is concluded that angioid streaks may be a manifestation of certain mesodermal tissue disorders, all of which manifest vascular abnormalities. The presence of angioid streaks in a patient demands a thorough investigation for underlying connective tissue disorders.

Angioid streaks are an uncommon phenomenon, usually seen bilaterally in the fundus oculi following ruptures in the elastic lamina of Bruch's membrane. Doyne (1889) first described these lesions in a patient who had received a 'poke in the eye'. He attributed them to a rupture of the pigment layer of the retina. Knapp (1892) first used the term angioid streaks. In the same year Plange (1892) also described the lesions in a separate article. Since that time there have been many reports in the literature describing angioid streaks in various disorders. Although they are most commonly associated with pseudoxanthoma elasticum, Paget's disease of bone and sickle cell anaemia, they have been described in many other conditions (Table 1).

This paper will review those conditions associated with angioid streaks and comment upon the significance of their interrelationships. While angioid streaks are most often seen by ophthalmologists, all physicians should be aware of the diseases in which they occur, since their presence may be the first clue to an underlying systemic disorder.

PATHOPHYSIOLOGY OF ANGIOID STREAKS

Angioid streaks usually are seen bilaterally in the ocular fundus following rupture in the elastic lamina of Bruch's membrane. They are seen on funduscopic examination as irregular bands or streaks coursing over both fundi. Characteristically they form an incomplete ring around the optic disc, and radiate from the disc toward the equator of the globe. The number of streaks may vary from one or two to a dense network, and their colour may vary from reddish-brown to dark brown or grey. During a casual examination they may be mistaken for retinal vessels. The lesion, however, lies beneath the retinal and above the choroidal vessels.

TABLE 1. Associated conditions

1. Pseudoxanthoma elasticum (Goodman *et al.*, 1963)
2. Osteitis deformans (Paget's disease of bone) (Paton, 1963; Terry, 1934; Morrison, 1941)
3. Sickle cell anaemia (Geeraets & Guerry, 1960)
4. Trauma (Doyne, 1889)
5. Ehlers-Danlos syndrome (Green *et al.*, 1966)
6. Hyperphosphataemia (McPhaul & Engel, 1961)
7. Acromegaly (Howard, 1963; Paton 1963)
8. Chromophobe adenoma (Wright, 1964)
9. Facial angiomatosis (Kalina, 1970)
10. Diffuse lipomatosis (Bonamour, 1959)
11. Dwarfism (Yoneyama & Yoneyama, 1960)
12. Epilepsy (Blobner, 1935)
13. Lead poisoning (de Simone & de Conciliis, 1958)
14. Idiopathic thrombocytopenic purpura (Yatzkan, 1957)
15. Neurofibromatosis (McWilliam, 1951)

The natural course of angioid streaks is quite varied. They may remain stationary or there may be periodic progressions in their length, breadth and number. While visual symptoms do not occur unless the streaks involve the macula, secondary complications are frequently associated with these lesions. They include hemorrhage into the macula, disciform degeneration of the retina, colloidal deposits on Bruch's membrane, choroidal atrophy and sclerosis, and progressive atrophy of the pigmentary epithelium (Duke-Elder, 1966; Hogan & Zimmerman, 1962; Trevor-Roper, 1942).

To understand the formation of angioid streaks and their association with many apparently unrelated diseases, it is necessary to understand the anatomy of Bruch's membrane. Bruch's membrane, or the lamina vitrea, is the thin membrane which lines the entire inner choroid. It was originally thought to be a two-layered structure consisting of an outer elastic lamina and an inner cuticular, collagenous layer. Electron microscopic studies have shown that it is a three-layered structure (Hogan,

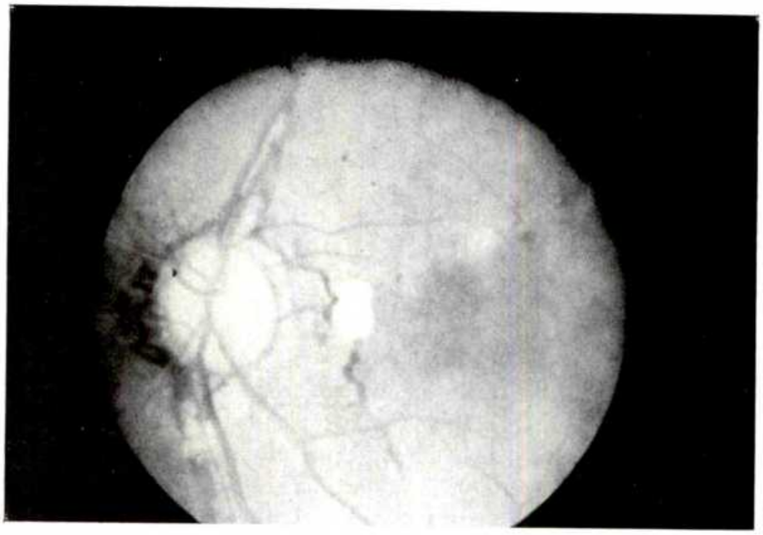

FIGURE 1. Fundus of a patient with pseudoxanthoma elasticum showing angioid streaks radiating out from the disc. Photograph courtesy of Dr C.J. Walsh.

1966). The innermost layer consists of collagen filaments and is adjacent to the retinal pigment epithelium. The second layer is composed of elastic tissue arranged around the circumference of the globe. The outermost layer is a second collagenous zone which adjoins the endothelium of the choriocapillaris.

The composition of Bruch's membrane renders it liable to the same diseases which affect collagenous and elastic tissues in other areas of the body. It has been established that the primary changes leading to the appearance of angioid streaks occur in the elastic portion of Bruch's membrane and in the choroidal vessels. In the membrane, the earliest changes affect the elastic layer where rupture occurs and where calcium may be deposited. The ciliary and choroidal arteries show thickening of the media and internal elastic lamina (Duke-Elder, 1966).

While angioid streaks have been described in a variety of diseases (Table 1), probably only pseudoxanthoma elasticum, osteitis deformans (Paget's disease of bone), sickle cell anaemia, and trauma are of clinical significance. The association with the other disorders is probably only coincidental.

Pseudoxanthoma elasticum

Pseudoxanthoma elasticum (PXE) is an hereditable disorder of elastic tissue which has manifestations in the skin, eyes and blood vessels. It was first associated with angioid streaks in the case described by Grönblad & Strandberg (1929). Since that time the combination of ocular and cutaneous pathology has been known as the Grönblad–Strandberg syndrome. This combination of angioid streaks and pseudoxanthoma elasticum in the same patient occurs with remarkable frequency. When looked for, angioid streaks have been found in more than 80% of patients with PXE. Similarly, 63% of the patients with angioid streaks have skin changes of PXE (Duke-Elder, 1966; Connor *et al.*, 1961; Paton, 1962; Percival, 1968).

Paget's disease of bone

Paget's disease of bone (osteitis deformans) associated with angioid streaks was first recorded by Verhoeff (1928). Later the association was mentioned by Rowland in an unpublished paper read before the New England Ophthalmological Society in 1929 (Rowland, 1934), and still later by Terry (1934). Other reports have been published since that time and all estimate that angioid streaks are present in from 8 to 15% of patients with Paget's disease of bone. The triad of osteitis deformans, angioid streaks and PXE has been reported in at least three cases (Shafer, Copelan & Beerman, 1957; Woodcock, 1952; Larmande & Margaillan, 1957).

Sickle cell anaemia

Paton (1959) published the first report of angioid streaks occurring in sickle cell disease. His two young male patients had sickle cell anaemia, but neither had osteitis deformans or PXE. Geeraets & Guerry (1960) reported on a series of sixty-nine sickleaemia patients in which five cases were associated with angioid streaks. Noteworthy was the fact that one of these individuals had PXE as well. These authors estimated that 6% of their patients with sickle cell anaemia had angioid streaks.

Ehlers-Danlos syndrome

Many ocular abnormalities have been observed in patients with Ehlers-Danlos syndrome. In a few cases, both angioid streaks and PXE have been found together in the same person. Green, Friedman-Kien & Banfield (1966) have published the only series of patients in which Ehlers-Danlos syndrome and angioid streaks coexisted in the absence of PXE. They studied 118 members of two different families and found ocular and cutaneous changes of Ehlers-Danlos syndrome in fifteen members. In two individuals from one family angioid streaks were observed.

Sporadic reports have appeared in the literature of the association of angioid streaks with other disorders. There is only one report (McPhaul & Engel, 1961) of heterotopic calcification, hyperphosphataemia and angioid streaks occurring in three patients, two of whom were brothers. These authors postulated that the heterotopic calcification is a secondary phenomenon occurring in patients with a *forme fruste* of PXE. Hyperphosphataemia is also a characteristic of active acromegaly, with heterotopic calcification in longstanding cases. Although rare, angioid streaks are seen in acromegaly (Howard, 1963; Paton, 1963). Paton (1963) has suggested that an abnormal facility for calcium deposition in Bruch's membrane exists in these patients, causing cracks to occur along the lines of force within the eye giving the typical picture of angioid streaks.

Recently, a case of facial angiomatosis with angioid streaks in the eye on the affected side of the face has been reported by Kalina (1970) who thought that there may have been small choroidal haemangiomas leading to calcification of Bruch's membrane and so to formation of angioid streaks, or there may be a genetic predisposition causing the formation of angioid streaks in the Sturge-Weber syndrome.

A case of angioid streaks and optic atrophy in a patient with severe gastrointestinal haemorrhage secondary to idiopathic thrombocytopenic purpura has been reported (Yatzkan, 1957). These ocular disturbances were thought to be secondary to the severe blood loss.

DISCUSSION

The observation that certain systemic disease processes are associated with angioid streaks in a significant number of cases has led to speculations about interrelated pathophysiologies. In pseudoxanthoma elasticum, for example, the elastic tissue of the skin, eye and blood vessels is abnormal. Histologically, there is clumping and calcification of the elastic fibres in the middle and lower dermis. Similar changes occur in the media and intima of blood vessels, in the elastic lamina of Bruch's membrane and in the endocardium (Scheie & Hogan, 1957; Goodman *et al.*, 1963). Vascular involvement may be manifested as early as the first decade with gastrointestinal haemorrhages, and claudication. Absent or diminished pulses in the extremities, hypertension, and myocardial infarction occur. While the basic elastic tissue defect is still not understood, electron microscopy has revealed that calcium deposits occur in the abnormal elastic tissue. The collagen fibrils appear normal. McKusick (1966) thought that the recessive mode of inheritance suggests an enzyme defect, and that a metabolic disorder injures the elastic fibres so that degeneration occurs. These degenerated elastic fibres are susceptible to secondary calcification regardless of their anatomical location.

Paget's disease of bone occurs in approximately 3% of people over the age of 40, and is rarely observed before 30 years of age. In this disease the organic matrix of bone, consisting largely of collagen, is involved. Extraskeletal calcification is common. There may be calcification in the media of arteries, Bruch's membrane and the skin. The calcium deposits found in Bruch's membrane may be responsible for the angioid streaks. Interestingly, these lesions are histologically indistinguishable from those found in PXE (Paton, 1962). In Paget's disease of bone, calcium deposits occur at sites of apparently *normal* elastic fibres whereas in PXE there is an *abnormality* of elastic fibres which facilitates calcification. This unique finding is as yet unexplained. It has been suggested, however, that Paget's disease is a disorder of connective tissue primarily involving the collagen matrix of bone (McKusick, 1966). Widespread abnormalities occur which might well lead to secondary calcification of Bruch's membrane and angioid streak formation.

Angioid streaks have been reported as occurring in 6% of cases of sickle cell anaemia (Geeraets & Guerry, 1960). On rare occasions sickle cell anaemia, PXE and angioid streaks have been reported together (Geeraets & Guerry, 1960; Seurig & Siefert, 1964). The occurrence of PXE and sickle cell anaemia without angioid streaks was discussed by Paton (1962) and by Charache & Richardson

(1964). The development of angioid streaks in sickle cell anaemia occurs secondarily to the impaired nutrition of Bruch's membrane which follows intravascular sickling and stasis. The nutritional damage which occurs in Bruch's membrane makes its fibrous elements very susceptible to rupture due to variations in ocular pressure.

Ehlers-Danlos syndrome is a rare generalized defect of connective tissue which is inherited as an autosomal dominant trait. The collagen and elastic fibres appear normal on electron microscopy. It was Jansen's impression (Jansen, 1955) that the basic defect is one of insufficient organization of collagen fibrils into bundles and of bundles into a strong meshwork; therefore, the collagen lacks its normal tensile strength. Since the fibres and fibre bundles can freely slide along each other, the skin is characteristically hyperextensible. Collagen itself is not extensible. Since angioid streaks have been described in only two patients with Ehlers-Danlos syndrome alone (Green *et al.*, 1966), it is difficult to draw any firm conclusions as to the aetiology or relationship between the formation of angioid streaks and the collagen organizational abnormality of Ehlers-Danlos syndrome.

CONCLUSION

At present there does not appear to be a pathogenesis common to angioid streaks and the disorders in which they most frequently occur. The defect in PXE has been shown to be in the elastic fibre, but the basis for this defect is still unknown. In Paget's disease, on the other hand, no defect has been demonstrated in the elastic fibre and the collagen matrix of bone is primarily involved (McKusick, 1966). In sickle cell anaemia there is no evidence of a generalized defect in connective tissue. Angioid streaks should be regarded as a sign of a possible generalized disorder of mesodermal tissue with widespread vascular effects. A thorough investigation should be undertaken to determine if any of the above-mentioned disorders are present.

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Eczema vaccinatum in a family with Darier's disease

O.P. SALO AND M.J. VALLE

Department of Dermatology, University Central Hospital, and Central Public Health Laboratory, Helsinki, Finland

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SUMMARY

Eczema vaccinatum in a mother and son with Darier's disease is described. The infection was accidentally transmitted from a recently vaccinated grandchild. Vaccinia virus was isolated from the blister fluid in both cases. The mother was treated with hyperimmune anti-vaccinial gamma-globulin and N-methylisatin β-thiosemicarbazone (Marboran^R). Both patients recovered.

Darier's disease is a rare, dominantly inherited skin disease, which is clinically characterized by brown hyperkeratotic papules coalescing into vegetating papillomatous papules, especially in the seborrhoeic areas. These plaques are at times eczematized and easily infected with bacteria, yeasts and viruses. Kaposi's varicelliform eruption caused by herpes simplex virus has been reported in a number of patients with Darier's disease (Bleiberg & Klosk, 1951; Hitselberger & Burns, 1961; Fisher & Kilbrick, 1963; von Weiss, Kilbrick & Lever, 1965; Doeglas & Moolhuysen, 1969; Izumi & Goldsmith, 1970). As far as we know, only two cases of Darier's disease complicated by eczema vaccinatum have been reported in the literature (Gerstein & Shelley, 1960; Loeffel & Meyer, 1970).

Before the introduction of anti-vaccinial treatment with either hyperimmune gamma-globulin (Kempe, 1960) or N-methylisatin β-thiosemicarbazone (Marboran^R), death was not uncommon among patients with eczema vaccinatum. Marboran has been shown to be able to inhibit the replication of poxviruses *in vitro* (Jocklik, 1966). It has also been shown to be effective in the prevention of smallpox in contacts of diseased patients (Bauer *et al.*, 1963). During the last 10 years several papers on the treatment of eczema vaccinatum with Marboran have been published (Turner, Bauer & Nimmo-Smith, 1962; Barlow, 1962; Mainwaring, 1962; Bauer, 1965; Webb, Marks & Reed, 1965; Adels & Oppé, 1966; Jaroszyńska-Weinberger, 1970).

In this paper we report eczema vaccinatum in a mother and son suffering from Darier's disease. The vaccinia infection originated from a family contact with a smallpox-vaccinated grandchild. The mother was treated with hyperimmune anti-vaccinial gamma-globulin and Marboran^R.

CASE REPORTS

Case 1

This 22-year-old man had had skin trouble as long as he could remember. He had never consulted a physician about it, but had noticed that it was similar to the condition from which his mother suffered (Darier's disease).

The patient's 2-year-old child was vaccinated against smallpox on 5 May 1969. On 21 May the patient developed a fever of 40°C. On 24 May he noticed rapidly enlarging vesicles on his chest, and on the back and scalp. He was admitted to the Department of Dermatology, University Central Hospital, Helsinki, on 27 May 1969.

On admission, he had brown hyperkeratotic papules on the scalp, chest, back and forearms, presenting the typical clinical picture of Darier's disease. In addition, there were purulent vesicles with a diameter up to 2 cm on the seborrheic areas of the chest, back and scalp. In the centre of most of the vesicles there was a brownish depression. Some of the vesicles were confluent, forming large weeping and crusted areas (Fig. 1). There was a narrow zone of erythema around the vaccinial lesions.

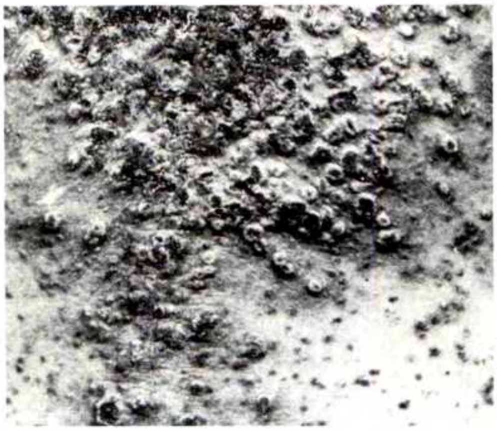

FIGURE 1. Case 1. Umbilicated, pustular, partly confluent lesions on the chest. Follicular keratoses of Darier's disease can be seen on the lower part of the picture.

Laboratory data. The blood count was normal with 6600 leukocytes/mm^3. The ESR was 22 mm/h and the urine was normal. From the weeping area of the chest an abundant growth of *Staphylococcus aureus* was obtained. Vaccinia virus was isolated from vesicle fluid on 6 June by the chorioallantoic membrane (CAM) technique in eggs. A great number of vaccinia virus pocks were obtained even in the first egg passage. Another vaccinia virus isolation was made by the same method on 18 June from serous exudate and crusts of a healing eczematous area. In this isolation no pocks were obtained in the first egg passage. However, the membranes were harvested, homogenized and a 'blind passage' was performed. Pocks were then obtained on the CAM and the virus was shown to be vaccinia virus. The additional identification of the virus was performed by a neutralization test in human amnion cell cultures with specific anti-vaccinia and anti-herpes simplex sera. The patient's vaccinia haemagglutination inhibition antibodies (HAI) rose from 1:128 on 28 May to 1:1024 on 4 June and fell to 1:256 on 20 June.

Treatment and course. The treatment consisted of tetracycline 1 g daily for 16 days because of the abundant growth of *Staph. aureus* from the infected areas. The local treatment comprised antibacterial compresses and ointments. The fever was already falling when the patient was admitted to hospital, and after the second day of hospital treatment his temperature was normal. When the patient was discharged from the hospital on 19 June 1969 his Darier's disease was much improved on the vaccinia infected areas.

Case 2

A 52-year-old woman, mother of case 1, had been treated for Darier's disease in the out-patient department of the Department of Dermatology, University Central Hospital, Helsinki, since 1937. A few days before admission she had taken care of her son and the vaccinated grandchild, living in the same household. On 28 May she was admitted to the ward for hospital treatment.

On admission she had typical Darier's disease, affecting the chest, back, face, behind the ears, the forearms, and the dorsa of the hands. On the forehead there were a couple of vesicles, and the skin behind the ears was weeping and crusted.

Laboratory data. On admission the blood count was normal with 6400 leukocytes/mm^3. The urine was normal. The ESR was 19 mm/h, rising to 43 mm/h on 23 June. Vaccinia virus was isolated on 6 June from blister fluid on CAM, and identified by the virus neutralization test. In the blood-samples taken on 2 and 13 June 1969, no HAI antibodies (<8) against vaccinia virus were found. Biopsy of the skin from a non-blistering papular lesion revealed changes typical of Darier's disease (Fig. 2).

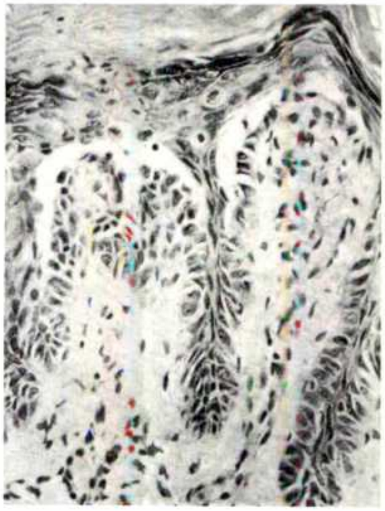

FIGURE 2. Case 2. Histology of Darier's disease with typical lacunae, corps ronds and grains.

Treatment and course. On 31 May the patient developed purulent blisters on the forehead, behind the ears and on the temples. At the same time large lymph nodes could be palpated on both sides of the neck. On admission the patient was afebrile, but within 5 days the temperature rose to 39·7°C (Fig. 3). On 2 June new vesicular lesions developed on the back and face, and some of the blisters became confluent (Fig. 4). There was grave facial oedema and the eyelids could not be opened. Concomitant with the development of fever and new skin lesions, leukocytosis of 13,600/mm^3 developed and the ESR rose to 58 mm/h. The vaccinial lesions disseminated rapidly, the vesicles were flat, and there was no erythema around them. Therefore treatment with 60 ml of human hyperimmune anti-vaccinia gamma-globulin and Marboran was commenced. The initial dose of Marboran was 200 mg/kg body-weight, and the treatment was continued with 50 mg/kg body-weight four times daily for 2 days (Bauer, 1967). On the fourth day after the institution of the treatment the patient became afebrile,

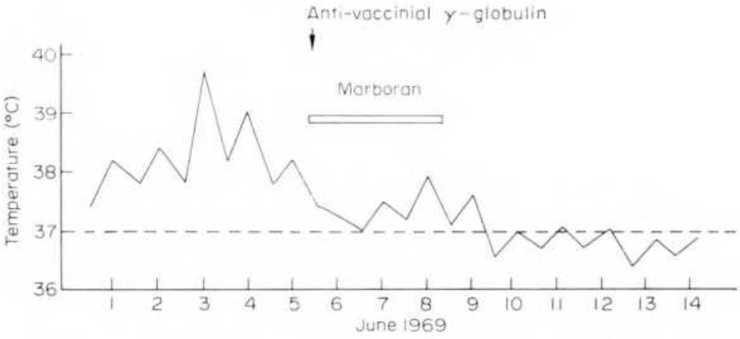

FIGURE 3. Case 2. Temperature chart and anti-vaccinial therapy.

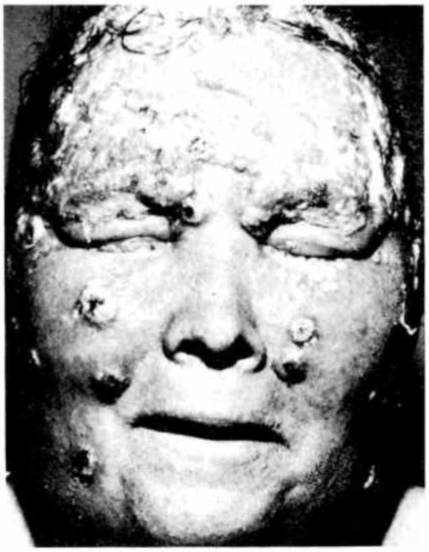

FIGURE 4. Case 2. Severe swelling of the face and umbilicated pustular lesions caused by vaccinia virus.

and the skin lesions started to subside. During Marboran treatment the patient had severe nausea but no vomiting.

Subsequent recovery from the disease was uncomplicated. The skin lesions healed rapidly, and on discharge from the hospital on 1 July 1969 the patient's Darier's disease was markedly better on the areas affected by the virus infection, whilst it had worsened on areas where there had been no vaccinial lesions.

DISCUSSION

Eczema vaccinatum is not a very common complication of smallpox vaccination. It occurred in about one case per 20,000 primary vaccinations in England and Wales in 1962. Most of the cases were patients with different forms of eczema (Copeman & Wallace, 1964). In an extensive series analyzed by Schroebler (1955) the frequency of eczema vaccinatum was only one case per 388,000 smallpox vaccinations. In the two previously published cases of eczema vaccinatum complicating Darier's disease, the vaccinial infection was accidentally transmitted from a recently vaccinated child (Gerstein & Shelley, 1960; Loeffel and Meyer, 1970). This indirect mode of infection was also the most common cause of eczema vaccinatum in the series of Copeman & Wallace (1964). The first of the present patients also acquired infection from his vaccinated child, and the second case represents an extension of the chain of infection to the mother of case 1; the three generations lived in the same household. All the findings discussed above strongly suggest that it is not sufficient merely to avoid vaccination of patients with active skin disease. It is equally important to take into consideration the possibility that there may be people in dermatological or other risk groups among the family contacts of the vaccinee.

Eczema vaccinatum is a life-threatening condition with a death rate in one series of 6% (Copeman & Wallace, 1964), and of 29·6% in another (Schroebler 1955). In view of this possibility case 2 was treated with both hyperimmune anti-vaccinial gamma-globulin and Marboran. Her disease was very severe with high and long-lasting fever, and rapid dissemination of the vesicles, both of which started to subside within 2 days of beginning the treatment. The absence of seroconversion in case 2 was somewhat unexpected, as case 1 produced very high HAI antibody levels. This fact may explain the more severe clinical course of case 2. Some defect in the immune mechanisms has been proposed to be responsible for the development of eczema vaccinatum (Ehrengut, 1966). The combined treatment of case 2 by hyperimmune gamma-globulin and Marboran seemed to have beneficial effect. It cannot be decided with certainty whether recovery was spontaneous or due to treatment. However, the extent of the skin lesions, flat vesicles without surrounding erythematous reaction, and the lack of antibody response, indicated very severe disease.

It has been reported that the lesions of Darier's disease improved on the virus infected areas after eczema herpeticum (Bleiberg & Klosk, 1951; Hitselberger & Burns, 1961; von Weiss *et al.*, 1965; Doeglas & Moolhuysen, 1969; Izumi & Goldsmith, 1970) and eczema vaccinatum (Gerstein & Shelley, 1960). This interesting phenomenon was observed also in the present patients. If Darier's disease were caused by a genetically transmitted viral infection, the clinical improvement might be due to viral interference, interferon, or some other mechanism increasing the host resistance (Wheelock, 1964; Armstrong *et al.*, 1970). At present, however, the clinical improvement of the symptoms of Darier's disease cannot adequately be explained.

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Comment

THERAPY OF FUNGAL INFECTIONS

Since the 1950s when griseofulvin provided the first breakthrough in the treatment of dermatophyte infections, and the polyene antifungal antibiotics in Candida and systemic fungal diseases, little advance had occurred until a few years ago, when the clinical applications of the antifungal properties of the very interesting drugs 5-fluorocytosine and clotrimazole were realized.

Griseofulvin still remains the most important therapeutic agent in the systemic treatment of dermatophyte infections and is specific for them. It is effective against all dermatophytes and is readily absorbed when given by mouth. It is of no value, however, against Candida infections. For these, the polyene antibiotics have, until recently, been the antifungal agents of choice. They are macrolide antibiotics produced mainly by *Streptomyces* species, which damage fungi by binding to sterol sites on the surface of susceptible organisms. Nystatin is probably the most widely used polyene in the dermatological field; it is very effective for local treatment, is not absorbed when taken by mouth and cannot be given parenterally. Amphotericin B is more active *in vitro* than nystatin and can also be used topically. However, unlike nystatin, amphotericin B can also be given by the intravenous route and is effective not only against yeasts but also against certain filamentous fungi causing deep mycoses. Pimaricin, another polyene, used topically for the treatment of Candida infections, is also active against the *Fusarium* species which can cause fungal keratitis. Two other polyene antibiotics, candicidin and trichomycin, are used mainly for the treatment of vaginal candidiasis, the latter also being active against *Trichomonas vaginalis*.

Hamycin, one of the more recently described polyenes (Thirumalachar, Menon & Bhatt, 1961), can be given by oral administration and shows a similar range of *in vitro* activity as amphotericin B. Most of the work on this antibiotic has been carried out in India and in the United States. There is, unfortunately, limited absorption of the drug, but it had been used with some success for the treatment of cases of North American blastomycosis. Side effects encountered were nausea, diarrhoea and anorexia. It is still being investigated and is not yet commercially available.

Neither primary resistance nor the development of resistant strains of fungi during therapy have presented problems during the often prolonged use of either griseofulvin or the polyene antibiotics. Unfortunately, both these types of resistance may occur to the antifungal compound 5-fluorocytosine. Nevertheless, the discovery of the antifungal properties of this pyrimidine derivative constituted a real advance in the treatment of fungal infections (Grunberg, Titsworth & Bennett, 1964). 5-Fluorocytosine, which is not metabolized at all by mammalian cells, is converted by susceptible fungi to 5-fluorouracil, which is highly toxic to the fungal cell by blocking the methylation of deoxyuridylic acid by thymidilate synthetase. The compound is soluble in water, well absorbed, relatively non-toxic, and can be given orally. It is fungistatic but becomes fungicidal *in vitro* when the time of contact is long. Its activity is primarily shown against the yeasts and it is not effective against the dermatophytes or filamentous fungi causing pulmonary or systemic infections, such as *Histoplasma capsulatum* and *Coccidioides immitis*. No *in vitro* activity can be demonstrated against the fungi causing subcutaneous mycoses apart from those responsible for chromoblastomycosis. There have now been a number of reports of the successful treatment of such cases by the oral administration of 5-fluorocytosine. Although it is possibly too early for there to have been sufficient follow-up of all these cases, the preliminary indications are that an acceptable form of treatment now exists for at least suppressing a disease that previously presented a very difficult therapeutic problem.

5-Fluorocytosine has been used primarily for the treatment of cases of disseminated candidiasis and of pulmonary and meningeal cryptococcosis. Where previously such cases had to be treated with intravenous amphotericin B with its possible associated nephrotoxicity, orally administered 5-fluorocytosine is now available which is well tolerated and gives very adequate antifungal concentrations in the blood, tissues and cerebrospinal fluid. Side effects which have been reported include reversible elevation of serum transaminases and also reversible pancytopenia. The normal recommended dose is 200 mg/kg body weight; caution is necessary if higher dosage is required since dose-related toxicity is to be anticipated with any anti-metabolite.

It must be stressed that when contemplating using 5-fluorocytosine, it is extremely important to test the sensitivity of the fungal strain isolated from the patient before treatment is started. Of 343 strains of *Candida albicans* isolated from clinical material, nearly 12% were shown to be resistant to 5-fluorocytosine (Hamilton-Miller, 1972). Any strain isolated during the course of treatment should also be tested to ensure that resistance has not developed. When carrying out such tests, medium free from peptones which contain pyrimidine and pyrimidine nucleosides must be used, as the *in vitro* activity of 5-fluorocytosine is known to be inhibited by these substances.

Although drug combinations have not in the past been used in the treatment of fungal infections, a report on the synergistic effects of amphotericin B and 5-fluorocytosine *in vitro* could well have clinical implications (Medoff *et al.*, 1972). As these authors pointed out, antifungal synergism may provide opportunities for more effective therapy by permitting the use of lower doses of both drugs. On the other hand, the increased potency of the combination may be counterbalanced by increased toxicity to host cells that would obviate their clinical usefulness. Enhanced *in vitro* antifungal activity of griseofulvin used with thiabendazole has been reported and this also may prove to be of clinical significance (Zaias *et al.*, 1972).

Clotrimazole is a tritylimidazole derivative (bis-phenyl(2-chlorophenyl)-1-imidazolylmethane) with a very broad spectrum of antifungal activity (Plempel *et al.*, 1970). Good fungistatic activity is obtained against the dermatophytes, *Candida* species, some fungi causing subcutaneous mycoses, Aspergilli, *Cryptococcus neoformans*, *H. capsulatum* and *C. immitis*. It is insoluble in water but readily soluble in lipids. It can be given orally in dosages which lead to inhibition of fungi causing human disease. Side effects reported include nausea, vomiting, depression and temporary psychotic changes. The drug is metabolized in the liver, and to detect early side effects regular liver function tests are necessary. From case reports it appears that clotrimazole probably disrupts certain normal liver enzyme activities. Decreasing serum concentrations of the drug after continuous administration have repeatedly been demonstrated, so that dosage may have to be progressively increased in order to maintain adequate blood levels. Its potential clinical value as an oral form of therapy remains uncertain. It has, however, been used successfully in the treatment of some types of candidiasis and aspergillosis, including ocular infections, and may prove of value for those cases where the causative organism has developed resistance to 5-fluorocytosine. There have been no reports of primary fungal resistance to clotrimazole nor of the emergence of resistance during treatment.

Whilst the development of a better-tolerated preparation of clotrimazole is awaited, or possibly the development of a related imidazole, a topical preparation (Canesten) is now available. Clinical trials have shown this to be as effective and as acceptable as Whitfield's ointment and tolnaftate (Tinaderm), for the treatment of dermatophyte infections of the skin, and as effective and acceptable as nystatin for the treatment of Candida infections of the skin and vagina. Such a spectrum of activity could be utilized when facilities for laboratory mycological diagnosis are unavailable.

Another imadazole derivative with a similar range of *in vitro* activity to that of clotrimazole is miconazole. This is a synthetic 1-phenethyl imidazole derivative which is only slightly soluble in water (Godefroi *et al.*, 1969; Van Cutsem & Thienpont, 1972). Miconazole was developed in Belgium, where the majority of clinical trials using a topical preparation have been carried out. It has been shown to be effective against both dermatophytes and Candida infections. Orally administered miconazole and intravenous drip-infusion of miconazole were found to be well-tolerated and effective against Candida infections. It is hoped that this interesting compound will soon become more freely available in this country so that another potential antifungal drug can be added to the small list of those already in use for the successful treatment of fungal infections.

YVONNE M. CLAYTON

Department of Medical Mycology
St John's Hospital
Leicester Square
London, W.C.2

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Correspondence

THE QUANTITATIVE DETERMINATION OF IMMUNOGLOBULINS BY RADIAL DIFFUSION TECHNIQUES

SIR, Examination of the literature shows that serum IgG, IgA and IgM levels are frequently determined in patients suffering from skin diseases, and that different workers often obtain different results in the same disease. Mancini's method (1965) and Fahey & McKelvey's method (1965) are commonly used for these determinations.

In this department, it was decided to introduce quantitative techniques for the above immunoglobulins and it seemed pertinent to compare the reliability of the two methods. Accordingly, specimens of serum were obtained from nine females and seven males who were not suffering from any disease in which these immunoglobulins could be expected to be altered. Duplicates of the specimens were obtained by dividing each into two before storage at −20°C in order that no sample or its duplicate was thawed more than once prior to its analysis, since it is well known that repeated freezing and thawing of serum proteins can profoundly affect their structure and properties. Each thawed sample was then subjected to the Mancini and the Fahey & McKelvey techniques, and each thawed duplicate was analysed, at a later date, in a similar way. In this way two groups of sixteen results for each technique were obtained. Hoechst 'Tripartigen' plates were used for the Mancini method and Hyland 'Immunoplates' for the Fahey & McKelvey method. In each method, the appropriate standards were used and the instructions issued by the manufacturers closely adhered to throughout. The results are shown in Table 1.

TABLE 1. Comparison of Mancini and Fahey & McKelvey methods

	IgG (mg/100 ml)	IgA (mg/100 ml)	IgM (mg/100 ml)	
			Females	Males
Mancini technique (no. of samples = 16)	1565 ± 88 1559 ± 85	292 ± 20 274 ± 21	260 ± 26 263 ± 25	211 ± 28 203 ± 28
Fahey & McKelvey technique (no. of samples = 16)	1775 ± 49 1607 ± 43	282 ± 23 281 ± 26	118 ± 8 131 ± 24	

Without the use of further statistical analysis it is obvious that the Mancini method is reproducible in all three determinations, whilst the Fahey & McKelvey technique is only reproducible for IgA. This lack of reproducibility may be due to the fact that in this method the diameter of the ring is measured before it has attained maximum size. Mancini, Carbonara & Heremans (1965) and Kalff (1970) have both shown that, at this stage, the size of the ring is still dependent upon the rate of diffusion of the antigen into the agar gel; this in turn is markedly dependent upon time and temperature.

The vast difference between the IgM results obtained by the two methods cannot be explained, although the manufacturers of the plates show this discrepancy in their literature.

It is concluded that the Mancini technique is superior to the Fahey & McKelvey technique; and this investigation demonstrates the necessity for stating which method is used in any determination of these immunoglobulins.

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F. Dorothy Dean

Department of Dermatology
University of Liverpool
Liverpool Royal Infirmary
Liverpool L3 5PU

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Book Reviews

Dermatology. An illustrated Guide. Lionel Fry (1973). London: Update Publications Ltd. Pp. 160, 420 colour illustrations. Price £4.75 + post and packing 25p. Not available through booksellers.

This book achieves its purpose in providing an illustrated guide to skin diseases. It is a splendid collection of 420 colour photographs which, with rare exceptions, give true colour reproduction and good definition of detail. As the publishers explain, it has been produced at its remarkably low price only because the pictures have been published previously in the journal *Update*. Only in one or two instances is it difficult to recognize what the picture is meant to show. Illustration 9 of guttate psoriasis is poor and should be replaced in future editions. Sometimes, and one can understand the temptation, Dr Fry has over-illustrated a very rare disorder, e.g. seven pictures of pemphigus.

The text which accompanies the illustrations gives a good account of the clinical features, the differential diagnosis and treatment of the disorders shown. No one would disagree with the emphasis the author places on taking a good history before examining the skin. No attempt is made to explain anatomy, physiology or aetiological mechanisms of disease. This simple approach will, I am sure, appeal to the non-dermatologist for whom the book is intended.

The advice on therapy is essentially conservative and well balanced. In his discussion of the management of psoriasis, ultraviolet light treatment is described as an in-patient facility, but many provincial centres now provide such treatment on an out-patient basis. To the mycological purist the retention of the term 'moniliasis' will cause surprise, but the superb photographs of candidiasis of the vulva will, I am sure, mitigate the offence.

In the chapter on parasitic infection, the scabies burrows are so well shown that even an acarus can be seen at the end of one. I have a preference for hunting her with a pin, and I felt she could be lifted off the page.

This work is intended for the non-specialist, and I can see it as an essential on the general practitioner's desk and in the casualty department of the hospital. It will also be of great value in teaching departments.

It is much easier to turn the pages of this beautifully produced book than to darken the room to show slides. Dr Fry and the Department of Audio-Visual Communication at St Mary's Hospital are to be congratulated.

IAN SNEDDON

Streptococci and Streptococcal Diseases. Recognition, Understanding and Management. LOUIS W.WANNAMAKER and JOHN MATSON (Eds.) (1972). New York and London: Academic Press. Pp. 635. Price £13.00.

This volume stems from a conference held in Minneapolis in 1971. It includes thirty-four contributions, many from distinguished bacteriologists and clinicians, and the discussions which followed the papers are printed, apparently in full. It is well edited, has a good index and will clearly be a useful source of information for all working in this area. For the clinical dermatologist this book cannot be listed as required reading, but for those whose interest lies in the microbiology of the skin there are many sections worthy of attention. Crause's introductory chapter 'The Streptococcal Cell—Relationship and Structure to Function and Pathogenesis', Kim and Watson's paper 'Streptococcal Exotoxins', Lancefield's 'Group A Streptococcal Infections in Animals, Natural and Experimental', and Gibbons' 'Ecology and Cariogenic Potential of Oral Streptococci', all include some material relevant to the dermatologist.

Amren in his paper, 'Unusual Forms of Streptococcal Disease', gives an excellent account of perianal cellulitis caused by Group A streptococci and its relationship to pharyngitis and vaginitis. He re-emphasizes perianal streptococcal carriage as a source of infections, and draws our attention to three patients he has observed with serious streptococcal infections arising from apparently trivial lesions on the hands. Two of these patients had abscesses between the pectorales muscles, one had septicaemia and two had streptococcal pleural effusions. All were afebrile but seriously ill. Dillon summarizes the current state of knowledge in the field of impetigo and nephritis, but most of the other clinical chapters are concerned with rheumatic fever, and nowhere is the relationship between streptococcal infection and guttate psoriasis or vasculitis discussed. Readers who, like this reviewer, find L-forms curiously fascinating, will be disappointed also to learn from Mortimer and his associates that, after exhaustive studies, they could find no evidence that L-forms act as persisting bacterial forms or are independently infectious in either cutaneous or systemic situations.

STEPHEN ROBERTS

Leishmaniasis tegumentaria americana. F.KERDEL-VEGAS (1972). Caracas: Biblioteca de la Academia de Ciencias Físicas, Matemáticas y Naturales de Venezuela. Pp. 135, illustrated.

Here is a masterly description of New World cutaneous leishmaniasis, covering all aspects of the disease—its history, epidemiology, pathology, clinical features and treatment. Written in Spanish, it covers 127 pages, in which are included 66 black and white photographs and microphotographs, and also a number of maps and diagrams.

Those accustomed to seeing cutaneous leishmaniasis in the Old World will be impressed by how similar are the clinical aspects of the disease in the two hemispheres, and this is hardly surprising in view of the fact that the causative parasites are identical morphologically and culturally and cause identical histological changes in the skin. To emphasize this point, of 30 clinical photographs of the disease as seen in Venezuela, only two would have to be excluded from a monograph on Old World cutaneous leishmaniasis—those of the progressive and mutilating form of muco-cutaneous leishmaniasis peculiar to the New World.

W.H.JOPLING

Clinical Dermatology. D.J. DEMIS, R.G. CROUNSE, R.L. DOBSON AND J. MCGUIRE (Eds.) (1972). Hagerstown, Maryland, New York and London: Harper and Row. 4 volumes and index volume (loose-leaf), profusely illustrated. Price £67.30. Annual supplements. Price £11.60.

It is the first duty of the reviewer of any book carefully to read the authors' or editors' preface and to base his assessment of their work on the extent to which they have achieved their declared objectives. Dr Demis and his colleagues in introducing their ambitious project claim: 'It provides a referenced, almost encyclopedic, source of information and a critical definitive discussion of all diseases of dermatologic interest or importance'. There can be no doubt that an 'almost encyclopaedic' and 'definitive' text, written in English, would be widely welcomed, for no such work has ever been attempted. There has never been in the English language any rival to Jadassohn's Handbuch and its remarkably accurate and comprehensive supplementary volumes. The three massive volumes on dermatology edited by Touraine for the Encyclopédie Médicochirurgicale provided a similar source book for those who read French: it was, like the present work, a loose-leaf production, kept up to date by regular supplements. Dr Demis and his team have made a brave effort, but 'Clinical Dermatology' is not in the same class as 'Jadassohn' or 'Touraine'.

Some of the contributions to this well produced book are excellent, but many are too brief and superficial to be regarded as 'near encyclopedic'. Some authors quote only the English language literature; many give less information and fewer references than some other current American, British, French and German textbooks, and some chapters are totally inadequate. The loose-leaf format should have given the editors exceptional freedom to ensure that all contributions were completely up to date, yet many chapters appear to have been written 3 or 4 years ago.

Many chapters devote a paragraph to the history of the condition with which they are concerned. Some knowledge of the history of a disease is often essential if modern concepts and nomenclature are to be understood. Many of the historical notes are clear, reliable and informative, but they are apparently not planned in the light of any consistent editorial policy. The minimum useful information must surely be one or two primary sources, with references, and the best secondary source. Bannister's disease is given as a synonym for angioedema, but his name does not occur in the brief 'history', which merely states (incorrectly) that Graver (*sic*) in 1848 made the first recorded mention of angioedema and (again incorrectly) that Quincke is credited with the classic description. No references are given either to Graves or to Quincke and the best secondary source, an excellent article by Schadeweldt, is not cited.

Dermatologists should try to persuade medical school libraries to buy this book because it contains much first class material and is superbly printed and illustrated, and also because this great project deserves the enthusiastic support of English speaking dermatologists. In many other specialties outstandingly good encyclopaedic texts in English are available; for example Duke-Elder's 'System of Ophthalmology', and the thirty volumes of the 'Handbook of Clinical Neurology', now in course of publication. In their preface the editors invite 'additions, comments or criticisms'. They ask for help 'to refine, correct and supplement the text'. They deserve to receive that help so that, through the intended annual supplements, 'a critical contemporary source book for our specialty' may be developed. It is to be hoped that English speaking dermatologists, who would like to see an English equivalent to 'Jadassohn', will give Dr Demis and his colleagues all possible assistance in the difficult task of compiling it.

ARTHUR ROOK

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Quarterly Review

THERAPEUTICS XXIII

Drug interactions. II

In a previous article (Bleehen & Edwards, 1973) the general principles concerned in drug interactions were discussed, together with possible sites of such encounters. Whilst such information provides an important background of scientific knowledge, it is no substitute for familiarity with individual drug idiosyncrasy. Because of this, it is proposed that the major known interactions of drugs commonly used in dermatological practice should be discussed in this and in further Reviews. It is important to note that only the more common practical problems can be outlined and that fresh interactions are continually being recognized, so that no article of this nature can be exhaustive.

ANALGESICS

The control of pain involves actions both at peripheral pain receptors and at the level of central cerebral appreciation. Narcotics in particular have actions at both levels and all analgesics will be potentiated by cerebral depressants, such as sedatives, tranquillizers, antihistamines, alcohol and anaesthetic agents (Goodman & Gilman, 1965, 1970).

Narcotic analgesics

Narcotic analgesics such as morphine and heroin are weakly basic, so that acidifying agents such as vitamin C will inhibit their action by increasing their urinary excretion, and alkalinizing agents will have the opposite effect (Milne, 1965).

Although in general it is the central depressant effect of morphine which is enhanced by antihistamines, cyproheptadine (Periactin) will reverse the analgesic effect (Stuart, 1968). Large doses of any cerebral depressant with a narcotic will result in respiratory depression, apnoea, hypothermia and coma (Goodman & Gilman, 1965, 1970).

Certain drugs with chemical structures similar to the major narcotics, such as nalorphine and pentazocine (Fortral), will reverse the central depressant effects. Since pentazocine is a powerful analgesic itself the analgesia is not greatly affected, but nalorphine will also reduce this effect of the narcotics (Bellville & Fleischli, 1968; Goodman & Gilman, 1965, 1970). A curious fact about pentazocine is that in over 50% of city dwellers a higher dosage of the drug is required, and this is also true of smokers. The reason for this phenomenon may be enzyme induction caused by atmospheric pollutants (Kerri-Szarto, 1970).

Propranolol, and perhaps other β-adrenergic blocking drugs, also enhances the cerebral depressant action of narcotics (Murmann, Almirante & Saccani-Guefi, 1966).

Thiazide diuretics sometimes cause postural hypotension, and this is increased by concomitant narcotic therapy (Goodman & Gilman, 1965, 1970).

Salicylates

The availability of these drugs is still uncontrolled, and they are responsible for a wide variety of adverse side effects and interactions, the main problem being gastric irritation. Alcohol increases both the severity and frequency of gastric haemorrhage (Goulston & Cooke, 1968), and indeed such potentiation occurs with other gastric ulcerogenic drugs, such as indomethacin (Jeremy & Towson, 1970), phenylbutazone (Azarnoff & Hurwitz, 1970) and corticosteroids (Stuart, 1968). Buffering of the salicylate may reduce this problem.

Acidification of the urine reduces renal excretion of aspirin and thus potentiates its effects. The converse is true of alkalinization of the urine, and this fact is used in the management of salicylate overdosage (Levy & Leonards, 1971).

Allopurinol and aspirin may often be taken together by a gouty subject. In normal doses salicylates interfere with the renal tubular excretion of uric acid and may also reduce the inhibiting effect of allopurinol on xanthine oxidase, so increasing serum levels. Clearly, the combined effects may induce renal tubular precipitation of urates (Miller, 1971).

Frusemide also interacts with salicylates at a renal level by competing for excretory sites; this may result in salicylate intoxication (Miller, 1971).

Both *para*-aminobenzoic acid (PABA) and *para*-aminosalicylic acid (PAS) potentiate salicylate, and the activity of the latter is itself enhanced (Salassa, Bollman & Dry, 1948; Stuart, 1968).

In addition to the effects mentioned above, corticosteroids enhance the rate of excretion of salicylates; hence reduction of the dose of steroids may result in salicylate intoxication (Klineberg & Miller, 1965). Steroids themselves are displaced from binding sites by aspirin and their anti-inflammatory effect is thus increased (Stuart, 1968).

Salicylates also have other interactions with indomethacin and phenylbutazone, in addition to potentiation of gastric haemorrhage. Indomethacin absorption is impaired in the presence of salicylate (Jeremy & Towson, 1970) and phenylbutazone competes both with aspirin and with uric acid for excretion by the renal tubules and so may precipitate gout (Oyer, Wagner & Schmid, 1966; Azarnoff & Hurwitz, 1970; Prescott, 1969).

The antimetabolites methotrexate and 6-mercaptopurine are potentiated by aspirin because of their displacement from protein binding sites and also by interference with metabolism of methotrexate. Serious pancytopenia may occur and be fatal (Dixon, Henderson & Rall, 1965).

The useful and toxic effects of both suphonamides and sulphonylureas (tolbutamide and chlorpropamide) are increased by aspirin because of displacement from binding sites. Salicylates also have an additive hypoglycaemic action at low dosage, but in certain individuals, paradoxically, high doses may cause hyperglycaemia (Anton, 1961; Stuart, 1968; Stowers, Constable & Hunter, 1959).

The anti-inflammatory effects of aspirin are antagonized by β-adrenergic blocking drugs (Riesterer & Jacques, 1968).

Indomethacin

Apart from the problems mentioned above, indomethacin also displaces coumarin anticoagulants from protein binding sites and may thus induce serious bleeding (Lubran, 1969).

Renal excretion of indomethacin may be reduced by probenecid and all its effects potentiated (Skeith, Simkin & Hedley, 1968).

Thyroxine replacement therapy seems to cause more cardiovascular side effects in the presence of indomethacin.

Phenylbutazone

Phenylbutazone, like salicylate, is acidic and causes gastric irritation. It also displaces oral anticoagulants from protein binding (Aggeler *et al.*, 1967). Long-term therapy, however, may result in a lessened anticoagulant effect due to enzyme induction (Hoffbrand & Kininmonth, 1967). Enzyme induction is also responsible for reduced effectiveness of antihistamines (Burns *et al.*, 1965), barbiturates (Conney *et al.*, 1960), oral contraceptives (Hartshorn, 1968), griseofulvin (Burns *et al.*, 1965) and corticosteroids (Hartshorn, 1968). Since phenylbutazone and barbiturates are both enzyme inducers there is mutual diminution of effectiveness.

Phenylbutazone increases the activity of several other drugs by displacement from serum protein binding. These drugs include oral antidiabetic agents (Prescott, 1969), insulin, phenytoin (Lucas, 1968), corticosteroids (Hartshorn, 1968), penicillin (Hartshorn, 1968) and sulphonamides (Hartshorn, 1968). The effect of displacement of corticosteroids is initally usually greater than that of enzyme induction. With long-term use, the balance of these different interactions may fall either way depending upon the steroid preparation.

Renal excretion of penicillin is reduced by phenylbutazone (Prescott, 1969).

Cholestyramine and tricyclic antidepressants, such as imipramine and desipramine, may inhibit intestinal absorption of phenylbutazone (Consolo, 1968; Gallo, Bailey & Schaffner, 1965).

Paracetamol

Paracetamol is fairly free of serious interactions, though it will displace coumarin anticoagulants from binding (Formiller & Cohon, 1969). Potentially lethal chronic poisoning may occur if it is prescribed with aminophenozone (Goodman & Gilman, 1965, 1970). Barbiturates reduce its effectiveness by increasing its metabolism through enzyme induction (Goodman & Gilman, 1965, 1970).

Combination of paracetamol and dextropropoxyphene (Distalgesic) produces analgesic activity superior to that of either drug alone.

SEDATIVES

Only the more commonly prescribed drugs in this section can be reviewed here.

Barbiturates

The major interactions of these drugs are due to microsomal enzyme induction which decreases the effectiveness of the following: coumarin anticoagulants (Burns, 1964), some analgesics (Formiller & Cohon, 1969), anticonvulsants (Clark *et al.*, 1967), some antihistamines (e.g. diphenhydramine, Clark *et al.*, 1967), corticosteroids (Clark *et al.*, 1967), oral contraceptives (Clark *et al.*, 1967), griseofulvin (Busfield *et al.*, 1963) and phenylbutazone. It is particularly important to avoid barbiturate night sedation whilst stabilizing a patient on anticoagulants in hospital, since the barbiturate is frequently discontinued when the patient returns home.

Alcohol potentiates the effects of barbiturates and can form a lethal combination with them. Paradoxically, alcohol may induce fits in epileptic patients normally controlled on barbiturates (Goodman & Gilman, 1965, 1970).

Barbiturates are weakly acidic, so that acidifying agents by depressing their ionization may allow the drug to pass through lipid membranes more easily. This increases both the amount absorbed from the gastrointestinal tract and the amount reaching the brain from the blood (Azarnoff & Hurwitz, 1970).

Tricyclic antidepressants potentiate barbiturates, and at the same time the adverse effects of tricyclics may become more pronounced. Imipramine may strongly potentiate thiopentone anaesthesia, and hypothermia may also result (Chappell, 1966; Dobkin, 1960). The tranquillizers all potentiate the sedative effects of barbiturates (Clark *et al.*, 1967), but chlorpromazine may reduce their anticonvulsant action (Sadore, Balagot & Reyes, 1956).

Barbiturate intoxication may result from their combined use with monoamine oxidase inhibitors

(MAOIs) because of inhibition of enzymes concerned with barbiturate metabolism (Domino, Sullivan & Luby, 1962). Thyroxine on the other hand increases barbiturate metabolism and reduces its effectiveness (Morelli & Melmon, 1968). Barbiturates potentiate oral antidiabetic drugs, and the sedative effect of barbiturates may be prolonged due to enzyme inhibition (Stuart, 1968).

The toxic effects of carbon tetrachloride are vastly increased in individuals taking barbiturates (Miller, 1971).

Dichloralphenazone

Dichloralphenazone (Welldorm) has a dual effect on oral anticoagulants, since a principal metabolite, trichloracetate, displaces them from protein binding whilst phenazone is an enzyme inducing agent. The net effect is a slight depression of anticoagulant activity. Alcohol has a marked and prolonged sedative effect when given either with chloral hydrate, 'Mickey Finn', or with Welldorm (Azarnoff & Hurwitz, 1970). Corticosteroid activity is also diminished because of enzyme induction (Hartshorn, 1968).

Nitrazepam

Nitrazepam seems to be the safest sedative, being free of known interactions apart from additive effects with other cerebral depressants.

Academic Division of Medicine — I. R. Edwards
University of Sheffield — S. S. Bleehen

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News and Notices

ROUTINE PATCH TEST SERIES (1974)

Recommendation by the International Contact Dermatitis Research Group

The International Contact Dermatitis Research Group (ICDRG) feel that it would be useful to publish a routine patch test series once a year in this journal. Deletions, additions and changes in concentration are based on the results of multicentre testing carried out by the Group during the year and reflect changes in the home and industrial environment (e.g. the recent inclusion of epoxy resins).

There is an obvious value in applying a standard set of allergens in every case, apart from any additional substances suggested by the history. By publishing these lists the Group feel that the most important allergens, at any rate for Western Europe, will be included and that reference to the 'Standard European Series' (or 'Battery') for any particular year will save much space in reporting results. It should be noted that this series differs from that recommended by the North American Contact Dermatitis Group (1973).

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Denmark:	Astra-Gruppen A/S, Roskildevej 22, DK-2620, Albertslund.
Japan:	Kaigar Gijutsu Koeki Co. Ltd, Show Building No. 43, Kanda-Konyacho, Chiyoda-ku, Tokyo.
Sweden:	IMECO Astra Agency Co. AB, S-151, 85 Södertälje.
U.K.:	Astra Chemicals Ltd, P.O. Box 117, Watford WD 17 QR.
U.S.A.:	Hollister-Stier Laboratories, North 3525 Regal St, Box 3145, Spokane 99220, Washington.
West Germany:	Firma Dr Friedrich Sasse, 78 Freiburg, Postfach 1423, DBR.

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Potassium dichromate	0·5	Petrolatum
Nickel sulphate	2·5	Petrolatum
Cobalt chloride	1·0	Petrolatum
PPD	2·0	Petrolatum
PPD mix*	0·6	Petrolatum
MBT mix*	1·0	Petrolatum
TMTD mix*	1·0	Petrolatum
Carbamix*	3·0	Petrolatum
Caine mix*	8·0	Petrolatum
Neomycin sulphate	20	Petrolatum
Chinoform	5	Petrolatum
Parabens*	15	Petrolatum
Wool alcohols	30	Petrolatum
Wood tars*	12	Petrolatum
Balsam of Peru	25	Petrolatum
Epoxy resin	1·0	Petrolatum
Turpentine peroxides	0·3	Olive oil
Colophony	20	Olive oil
Formaldehyde	2·0	Water

* The individual ingredients of these 'mixes' can be found in the 'Trolab' list of allergens.

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PPD	PPD mix
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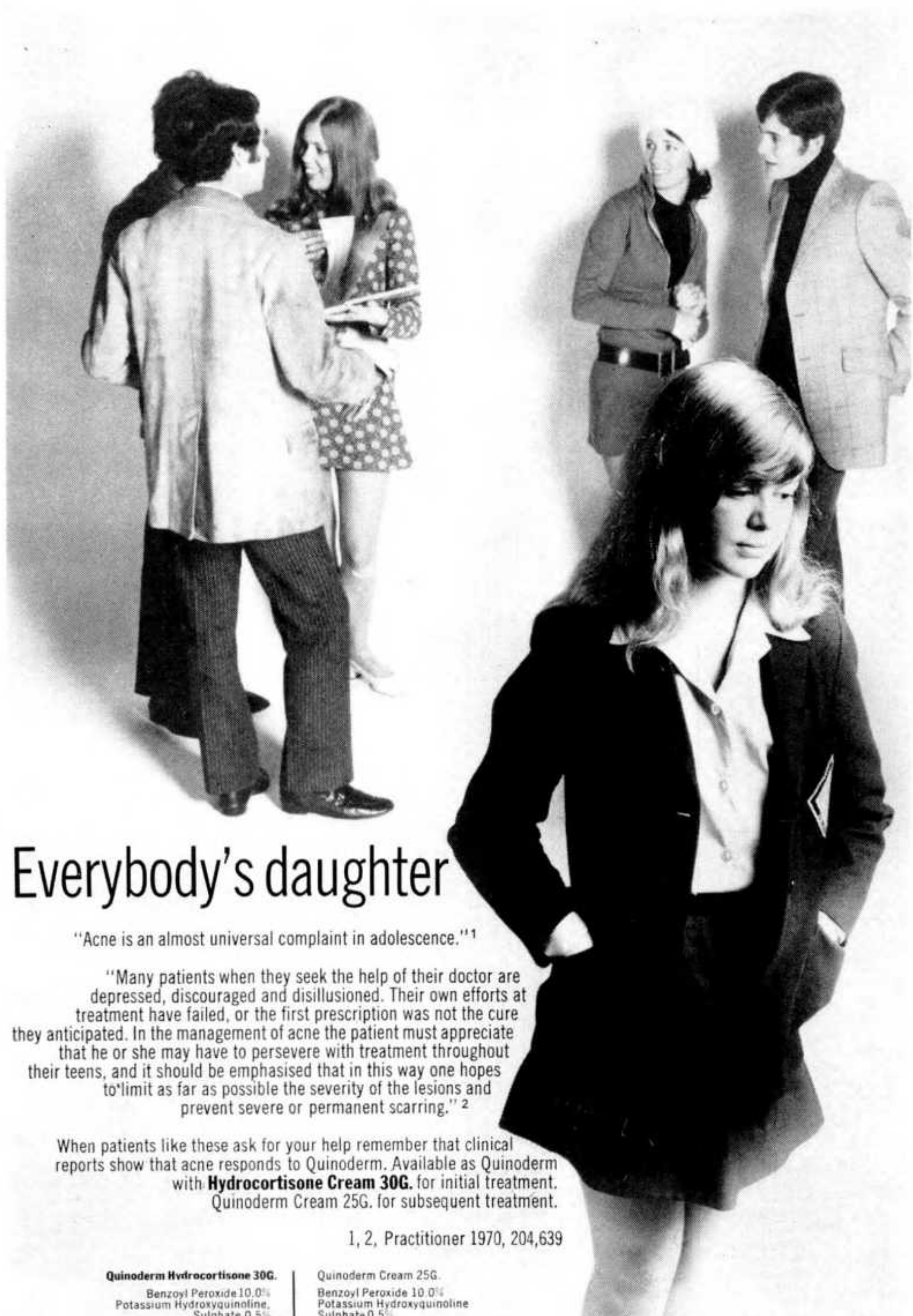

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2. CUTIS (Yorke Medical Group) (1972), 9:77

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Textbook of Dermatology

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F.J.G. Ebling D.SC. PH.D.

The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this editon is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

List of Contributors

- Harvey Baker M.D.(Leeds) F.R.C.P.(Lond.)
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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	percent	%
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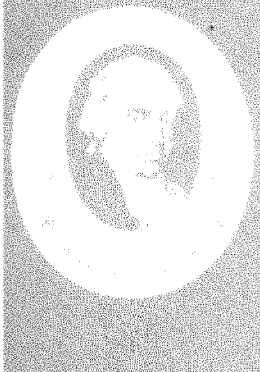

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Carol Fisher keeps herself very much to herself

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Have you ever stopped to think what it is really like to be a psoriatic? Psoriasis is more than a skin disease. It is a terrible mental affliction, particularly for young people and can literally ruin their lives. Most forms of treatment are equally depressing.

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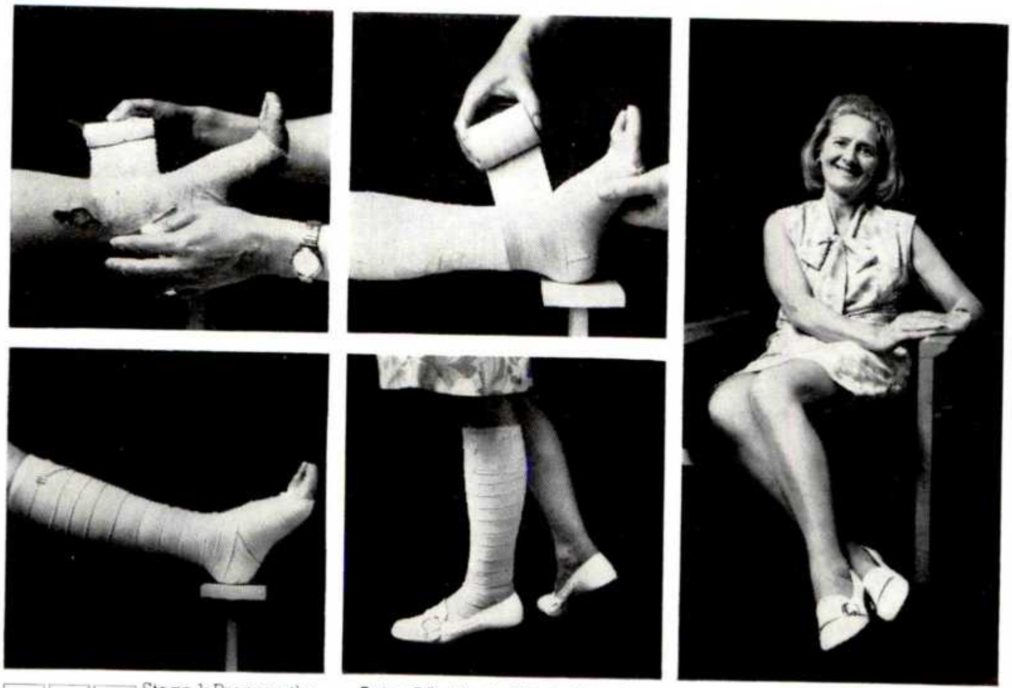

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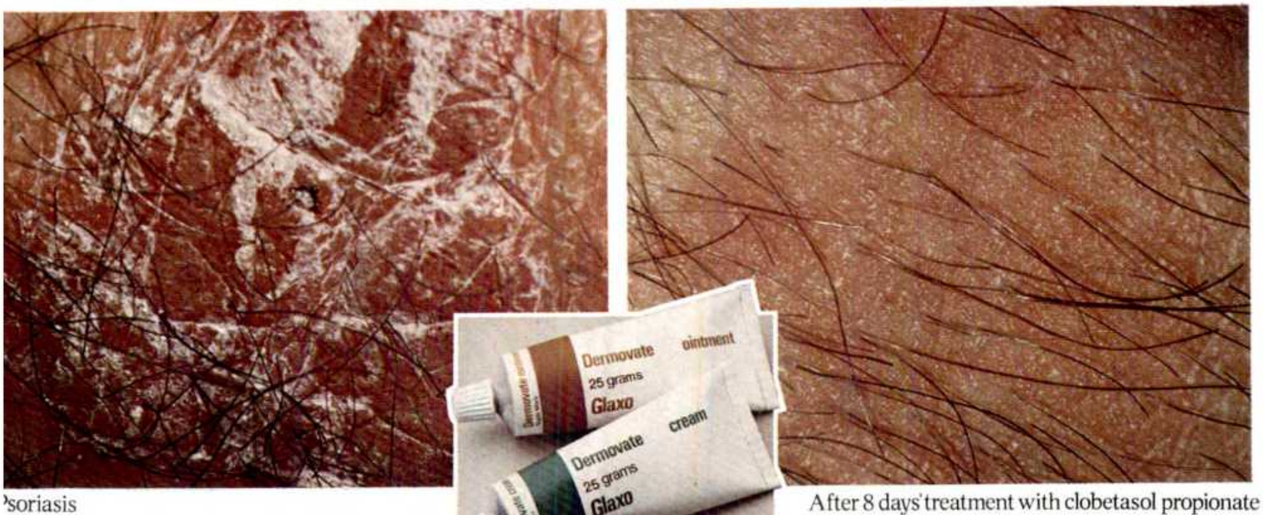

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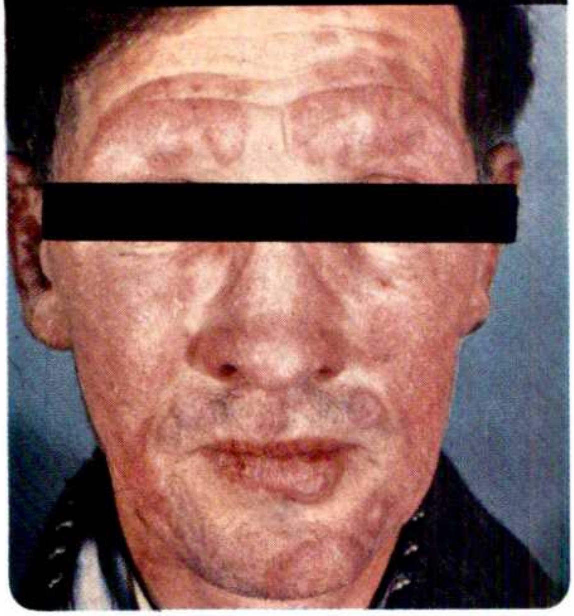

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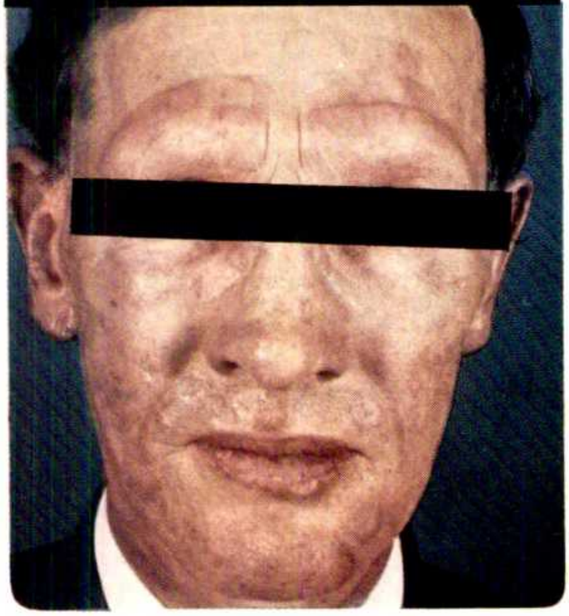

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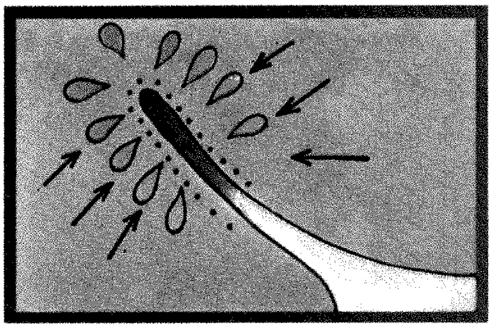

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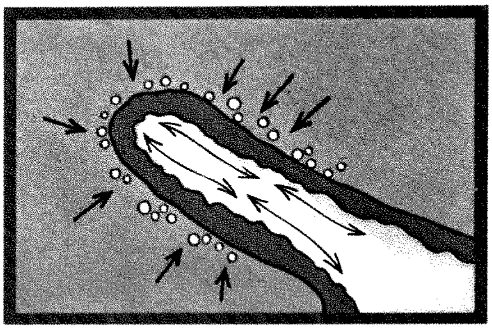

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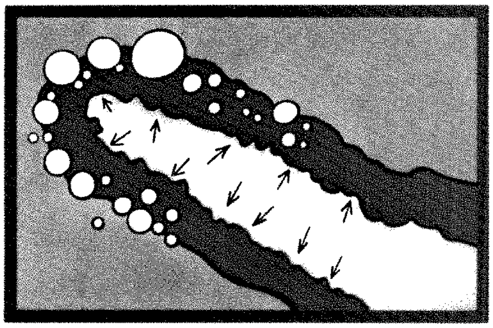

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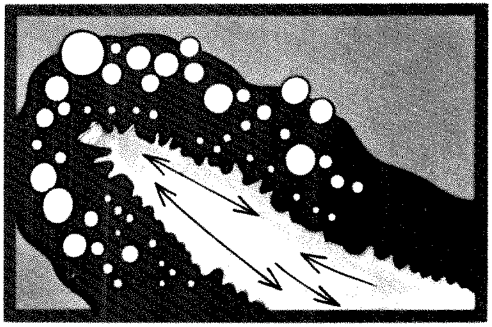

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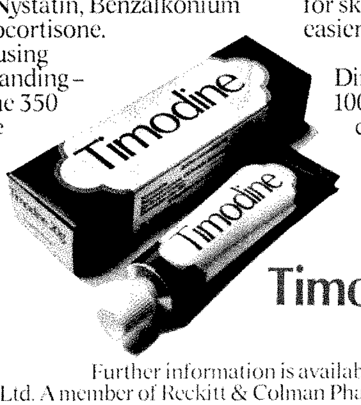

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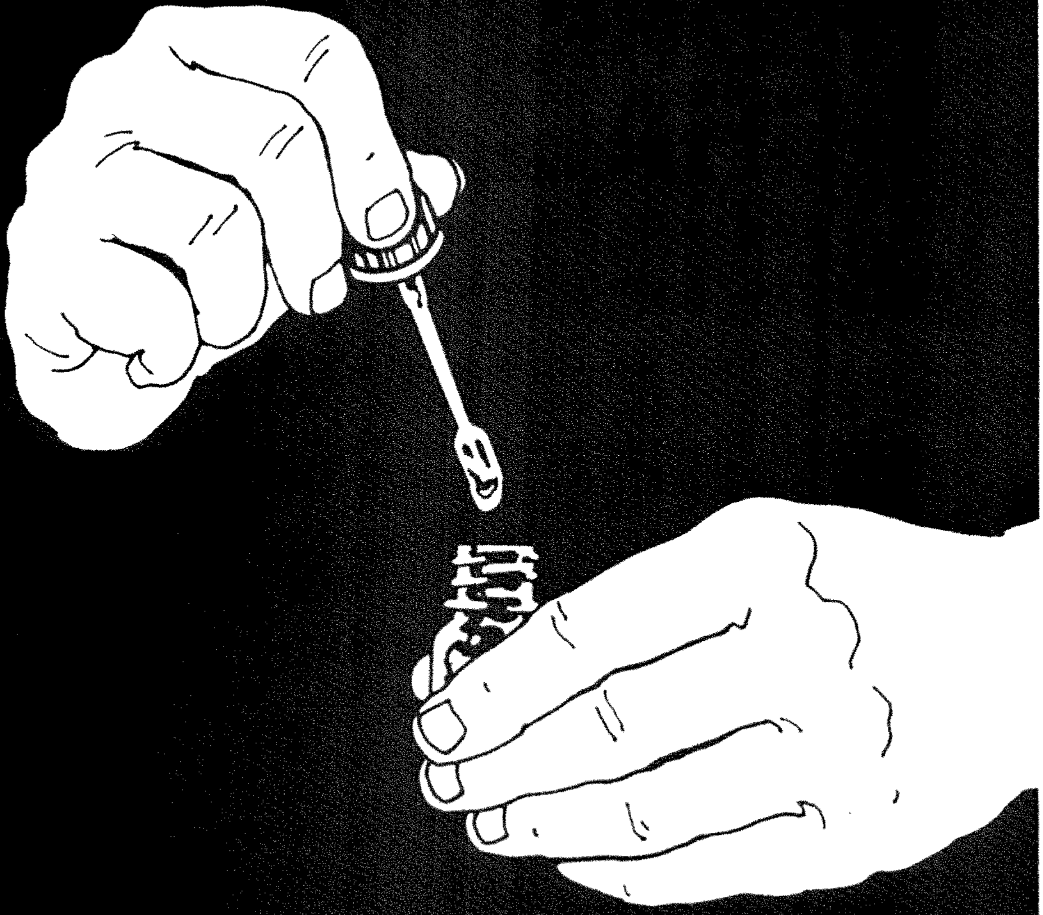

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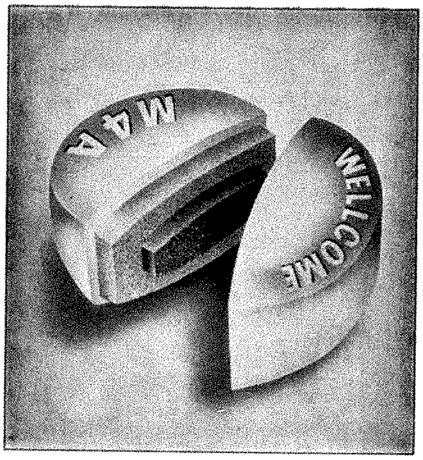

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Clinical and Laboratory Investigations

Oral lesions in dermatitis herpetiformis

N.G.FRASER, N.W.KERR AND D.DONALD

Departments of Dermatology and Dental Surgery, Aberdeen Royal Infirmary,
and Department of Pathology, University of Aberdeen

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SUMMARY

The oral mucosa was examined in fifteen patients with active dermatitis herpetiformis. The lesions seen were classified as erythematous, pseudo-vesicular, purpuric, and erosive in type. The only symptom experienced was of a painful, burning sensation in lesions on the tongue and upper alveolar ridge in two patients. The distribution of lesions, predominantly on the buccal mucosa adjacent to the occlusal plane or beneath existing dentures, suggested that trauma was a factor in their pathogenesis. Histologically, the features noted were similar to those occurring in skin lesions, except that sub-epithelial microvesicle formation was seen in only one of nine biopsies, and that purpura was a predominant feature. Also, the presence of granular IgA deposits in the submucosal papillae of early lesions was similar to the reported skin findings with the direct immunofluorescence technique. Oral lesions were found in over 70% of patients examined; an incidence much higher than previously supposed.

Review of clinical studies in dermatitis herpetiformis suggests that oral involvement is uncommon. Thus, Smith (1966) reported mouth lesions in only one of 149 patients, and Lodin & Stigell (1965) stated that mucosal involvement was almost invariably absent. Some authors, indeed, failed to comment on oral lesions (Björnberg & Hellgren, 1962; Evans & Fraser, 1963) while Tolman, Moschella & & Schneiderman (1959) used their absence as a criterion for diagnosis of the disease. Similarly, dermatological textbooks state either that oral involvement in dermatitis herpetiformis does not occur (Lever, 1967), or that it is rare (Pillsbury, Shelley & Kligman, 1956; Domonkos, 1971; Sneddon, 1972).

The present investigation was undertaken to examine the oral mucosa in patients with active dermatitis herpetiformis, and to study the observed abnormalities with conventional histological and immunofluorescence techniques.

PATIENTS AND METHODS

Fifteen patients (fourteen adults and one girl aged 16) with dermatitis herpetiformis were studied (Table 1). The diagnosis was confirmed by skin biopsy in all patients.

The disease was controlled in twelve patients on dapsone therapy; in one on sulphapyridine; and in one on both dapsone and sulphapyridine. An exacerbation of disease activity was induced in these patients by stopping treatment. Nine patients were examined 3 or 4 days after treatment was stopped; three patients at 5, 6, and 10 days respectively; and in two patients an exacerbation of disease activity did not occur for 3 and 4 weeks respectively. In the remaining patient the disease was active while on dapsone therapy, and did not significantly increase in severity when the drug was withdrawn. In two patients treatment was stopped on two and three occasions respectively. The skin disease activity was graded in the following manner: 4+, active, with bullae; 3+, active, with vesicles; 2+, moderately active, with or without minimal vesiculation; 1+, minimally active, with few lesions; 0, no active lesions present.

Oral examination

This was carried out in a dental surgery, and abnormalities photographed. The probable duration of oral changes was dated from the time of exacerbation of the skin eruption. Fifty patients attending the dermatological out-patient department with miscellaneous skin diseases served as controls: the oral mucosa was examined and abnormalities noted.

Histological studies

In nine patients one of the oral lesions was biopsied. Seven specimens were fixed in 10% neutral buffered formalin, and paraffin embedded sections (7 μm thick) were prepared, stained with haematoxylin and eosin, and examined in the light microscope: specimens of buccal and gingival mucosa from a normal control subject were similarly treated. The two other specimens were snap frozen for immunofluorescence studies as described below, and in these, light microscopy was carried out on frozen sections (7 μm thick) stained with haematoxylin and eosin.

Immunofluorescence studies

In four patients biopsy material was snap frozen by placing it on the inner surface of a universal container immersed in a mixture of solid CO_2 snow and ethanol. Cryostat sections (7 μm thick) were post-fixed with acetone and incubated at room temperature for 30 min with monospecific fluorescein-labelled sheep anti-human IgG, anti-IgA and anti-IgM (Wellcome Reagents Ltd, Beckenham, England). After washing in phosphate-buffered saline (pH 7·2) for 10 min, the sections were mounted in buffered glycerol and examined in a Zeiss microscope with a mercury super pressure lamp (HBO 200 W), exciter filter UG1 and barrier filter 50. Buccal and gingival mucosa from a normal control subject were similarly examined.

RESULTS

Clinical findings

Details of the fifteen dermatitis herpetiformis patients are given in Table 1.

Types of oral lesion

Four main types of lesion were noted:

(*i*) *Erythematous.* Macular and papular varieties occurred. Macular lesions were 1–3 mm in diameter and blanched on pressure (Fig. 1): they were noted in five patients (lips, three; buccal mucosa, one; soft palate, one). Papular lesions occurred in two patients: on the tongue (Fig. 2) and right anterior fauces respectively.

(*ii*) *Pseudo-vesicular.* This consisted of a pale or white area of mucosa, 1–3 mm in diameter, which was often surrounded by an erythematous zone (Fig. 3). The pale area suggested incipient vesicle formation, but even in older lesions no recognizable vesicle formation occurred (Fig. 4). Purpuric

TABLE 1. Oral manifestations in fifteen patients with active dermatitis herpetiformis

			Types of oral lesion				
			Erythematous				
Patient	Sex and age	Disease activity	Macular	Papular	Pseudo-vesicular	Purpuric	Erosive
1 (a)*	M 42	3+	−	+	−	+	−
(b)*		3+	−	+	+	+	−
2	M 65	3+	−	−	−	+	−
3	M 62	2+	+	−	−	−	−
4	M 56	3+	+	−	+	−	−
5 (a)*	F 16	3+	+	+	+	−	−
(b)*		3+	−	−	+	−	−
(c)*		3+	−	−	+	+	−
6	F 59	3+	−	−	−	−	−
7	M 65	2+	−	−	−	−	+
8	M 69	3+	−	−	−	−	−
9	M 61	3+	−	−	+	−	−
10	M 71	2+	−	−	−	+	−
11	F 51	3+	+	−	−	+	−
12	M 48	2+	−	−	−	−	−
13	M 38	2+	−	−	−	−	+
14	F 30	2+	+	−	+	−	+
15	F 35	1+	−	−	+	+	−

* Patients examined on separate occasions after dapsone withdrawal.

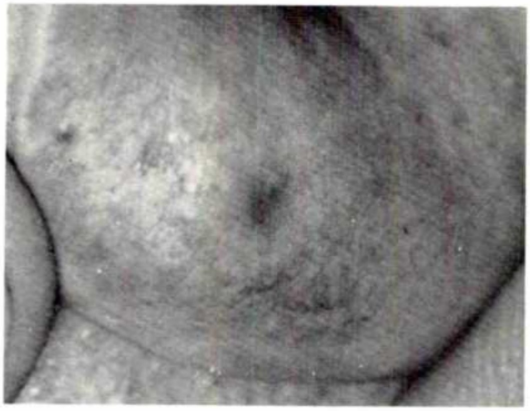

FIGURE 1. Erythematous lesion of dermatitis herpetiformis on mucosa of lower lip.

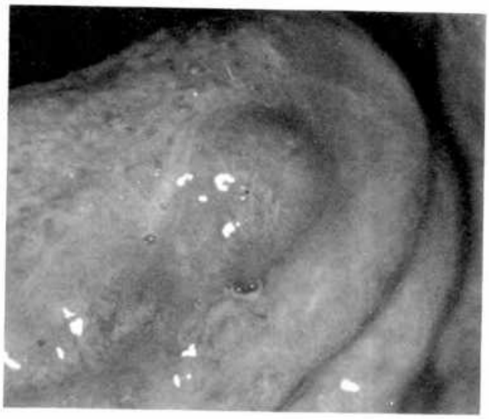

FIGURE 2. Papular lesion of dermatitis herpetiformis on right lateral border of tongue near the tip.

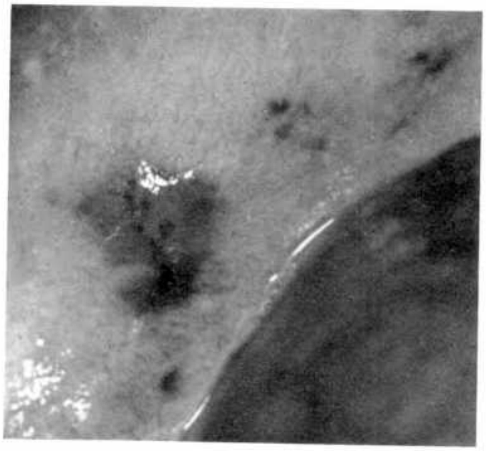

FIGURE 3. Early pseudo-vesicular lesion of dermatitis herpetiformis in the right buccal sulcus opposite the 2nd molar, showing a pale central area, erythematous flare and purpura: it is surrounded by a number of smaller lesions.

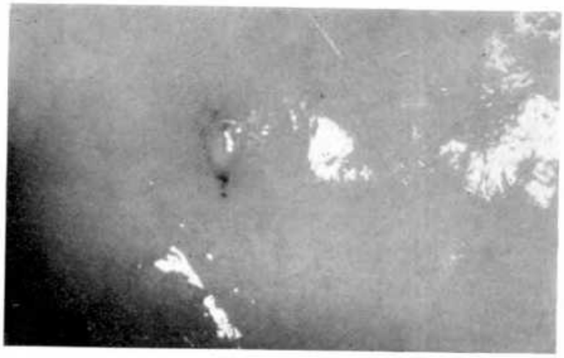

FIGURE 4. Pseudo-vesicular lesion of dermatitis herpetiformis of left buccal mucosa, on level of occlusal plane opposite 2nd molar, consisting of pale white central area with minimal surrounding erythema and purpura.

elements occurred in and around the pale areas in six of the eight lesions examined (Figs. 3 and 4). In patient 5a, two adjacent pale areas were noted on a single erythematous base.

The lesions in this category all occurred on the buccal mucosa at a level coinciding with the occlusal plane of the teeth, except in one patient who had lesions on the tongue and upper alveolar ridge.

(*iii*) *Purpuric.* Two varieties of this type of lesion were noted: in the first, grouped purpuric punctae, with pale mucosal areas intermingled and minimal surrounding erythema, were seen (Fig. 5); and in the second, discrete dark erythemato-purpuric patches 1–10 mm in diameter occurred (Fig. 6). The first variety of purpuric lesion was present on the lower gum under dentures in two patients; and the second variety appeared in the area of junction of the hard and soft palate in three patients, and on the buccal mucosa at the level of the dental occlusal plane in two.

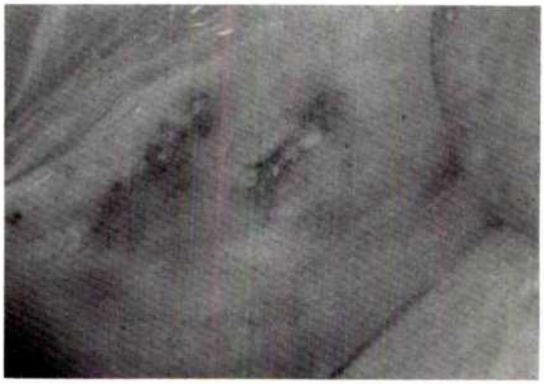

FIGURE 5. Purpuric lesion of dermatitis herpetiformis on alveolar crest in lower right 2nd–3rd molar region: purpura seen with some intermingled pale areas.

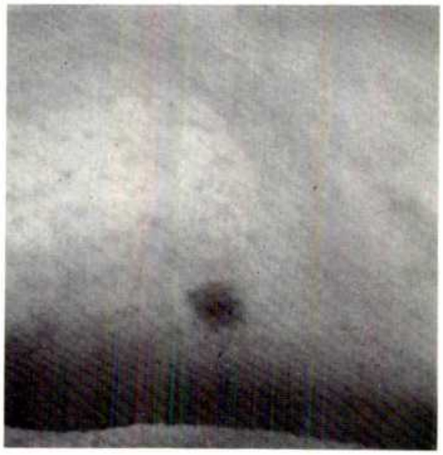

FIGURE 6. Purpuric lesion of dermatitis herpetiformis present 1 cm distal to junction of hard and soft palate on left side of midline.

(*iv*) *Erosive.* This type of lesion was characterized by small pale yellow areas surrounded by a zone of erythema (Fig. 7). Purpuric elements were present in the erythematous zones in two of the three

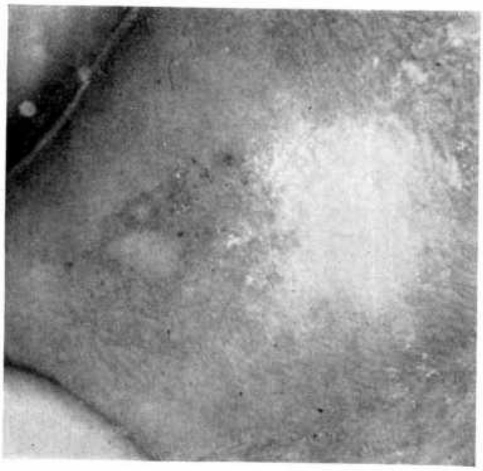

FIGURE 7. Erosive lesion of dermatitis herpetiformis on mucosa of lower lip.

patients affected. The lesions were sited on the lower lips in two patients, and on the upper alveolar ridge beneath a denture in the other patient.

Isolated purpuric punctae were seen in seven of the fifteen patients: buccal mucosa, three; alveolar ridge, three; lips, two; soft palate, one; anterior fauces, one.

Symptoms

Two patients complained of a painful, burning sensation in lesions: patient 5a in relation to the papular tongue lesion (Fig. 2); and patient 15 in relation to small pseudo-vesicular lesions on the tongue and alveolar ridge. Both these patients had previously noted painful mouth lesions when the skin disease was active. In all other patients studied the lesions were asymptomatic; and there was no relevant past history of mouth lesions.

Findings in control subjects

In twenty of the fifty control subjects examined, purpuric punctae, usually of pin-point size, were noted: in seventeen subjects they were on the buccal mucosa at the level of the occlusal plane; in two subjects on the alveolar ridge beneath dentures; and in one subject on the soft palate. Eleven of the seventeen subjects with lesions on the buccal mucosa wore full upper and lower dentures. Small keratinized linear areas on the buccal mucosa at the level of the occlusal plane were found in five subjects. In two subjects small maculo-erythematous patches occurred on the hard palate. Inflammatory but symptomless hypertrophic areas under upper dentures, a recognized effect of ill-fitting dentures, were seen in eleven subjects.

Histological studies

Papillary micro-abscesses, consisting of neutrophil polymorphonuclear leukocytes, were present in all nine biopsies (Fig. 8): leukocytoclasis accompanied these changes in all but one biopsy. In the two patients with erosive lesions, micro-abscesses were seen adjacent to the ulcerated areas (Fig. 9). Separation of the epithelium from the submucosa by early vesicle formation occurred in only one specimen, and the microvesicles formed contained a large number of red blood cells in addition to polymorphs: the submucosal oedema noted in four others was minimal. Epithelial oedema, however, was present in seven biopsies, and in two of these epithelial tears were also noted (Fig. 8).

The submucosal infiltrate consisted predominantly of neutrophils with scanty eosinophils and lymphocytes, and was diffuse rather than perivascular in seven of the nine specimens. A prominent feature in seven of the nine oral lesions examined was the presence of extravasated red blood cells: in six they were scattered diffusely throughout the submucosa; in five they aggregated subepithelially adjacent to papillary micro-abscesses (Fig. 8), or as part of the micro-abscesses (Fig. 10); and in four red blood cells were present in the epithelium (Fig. 10).

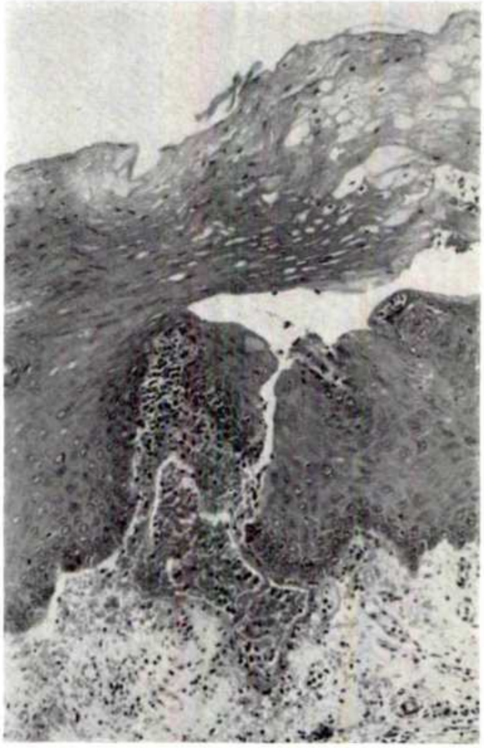

FIGURE 8. Pseudo-vesicular oral lesion of dermatitis herpetiformis. Papillary micro-abscess containing neutrophil polymorphonuclear leukocytes. Aggregate of RBCs with surrounding fibrin deposition at epithelial/submucosal junction. Overlying epithelium shows oedema of cells and splitting (× 112).

Fibrin deposition was noted in one early lesion in dermal papillae in which only minimal cellular infiltration had occurred. Otherwise, fibrin deposition was seen only in the ulcerated lesions and in one patient around a haemorrhagic area (Fig. 8).

No significant changes were seen in the small submucosal blood vessels.

Correlation of clinical and histological findings

The nine biopsies were obtained from different types of clinical lesion—pseudo-vesicular, three; purpuric, with pale mucosal areas intermingled, two, and without these areas, two; erosive, two—and an attempt was made to correlate the histological changes with the clinical appearances.

The clinically pale areas seen in the pseudo-vesicular and purpuric lesions (Figs. 3, 4, and 5) appeared histologically to be represented by epithelial oedema, which in two specimens was accom-

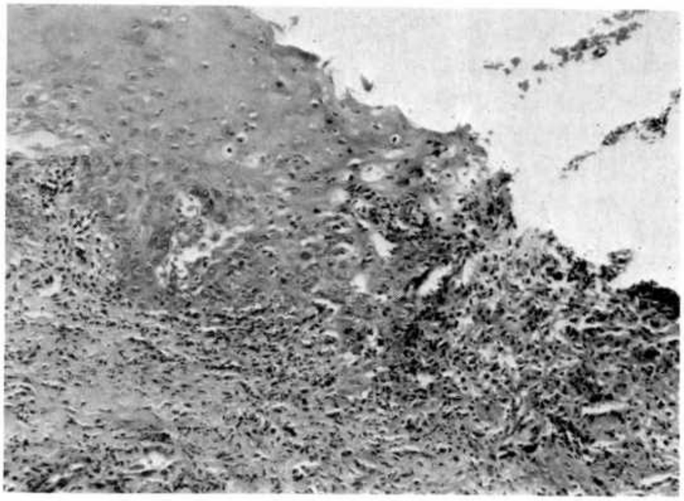

FIGURE 9. Erosive oral lesion of dermatitis herpetiformis. Ulceration of epithelium with a predominantly neutrophil infiltrate in the submucosa and ulcer floor. A small papillary micro-abscess is present adjacent to the ulcer on the extreme left of the field (× 112).

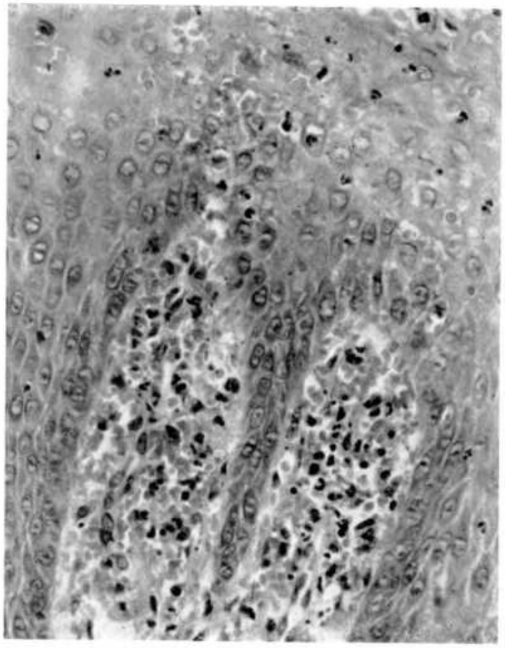

FIGURE 10. Purpuric oral lesion of dermatitis herpetiformis. Infiltrate of RBCs in papillary micro-abscesses and in epithelium (× 280).

panied by epithelial splitting (Fig. 8). These pale areas could not be correlated with vesicle or incipient vesicle formation, since in only one purpuric lesion on the gum did fluid accumulate in relation to papillary micro-abscesses, and in that specimen the micro-abscesses consisted predominantly of red blood cells: nor could the colour change have been markedly influenced by diffuse submucosal oedema, since this was minimal and infrequent. More predictably, the discrete purpuric punctae, which were a feature of many lesions, were seen histologically to consist of aggregates of red blood cells at the epithelial/submucosal junction (Fig. 8); and the dark erythematous colour of some lesions (Fig. 6) was due to the presence of large numbers of free red blood cells in the tissues and throughout the epithelium (Fig. 10). Finally, accumulation of leukocytes and fibrin in the floor of the ulcers (Fig. 9) produced the yellow colour of the erosive lesions (Fig. 7).

Immunofluorescence studies

In the two early lesions (<24 h old) examined, granular deposits of IgA were demonstrated in the basement membrane area of the submucosal papillae, and in the submucosa, particularly in the walls of blood vessels (Fig. 11). In one of these biopsies IgG deposits were seen also in the submucosal papillae. Immunoglobulin deposits were not demonstrated in the two late lesions (probable duration 2 and 3 days respectively), nor in the two control biopsies.

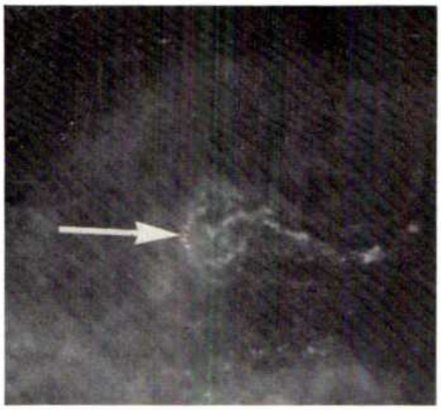

FIGURE 11. Early oral lesion of dermatitis herpetiformis. Direct immunofluorescence technique. Sheep anti-human IgA. Note granular deposits of IgA at the epithelial/submucosal junction and in the upper submucosa (× 160).

DISCUSSION

In their review of diseases affecting the oral mucosa, Schuerman, Greither & Hornstein (1966) discussed the oral manifestations of dermatitis herpetiformis. They stated that the prevalent lesion was the vesicle which tended to rupture quickly leaving ulceration; that the ulcers which formed were prone to bleed and healed slowly on treatment; and that symptoms of pain or a burning sensation might accompany the lesions. The histological appearance described was a subepithelial tension vesicle, not considered to be diagnostic of dermatitis herpetiformis. Smith (1966) also reported small oral blisters occurring on one occasion in a patient with dermatitis herpetiformis; and Schimpf (1964) described a patient in whom blistering of the oral mucosa had been the presenting feature of the disease. Dermatological textbooks, however, which admit the occurrence of oral lesions in this disease, generally fail to comment on the clinical features of the lesions (Pillsbury *et al.*, 1956; Domonkos, 1971; Graham, 1971; Sneddon, 1972).

In the present investigation, a number of different types of clinical lesion were noted in the mouth in dermatitis herpetiformis, and an attempt was made to classify these lesions (Table 1). Strict separation of types, however, is somewhat artificial, as in four of the six patients with pseudo-vesicular lesions purpura was also observed, and in two of the six patients with the purpuric type of lesion some areas of pallor of the mucous membrane, suggestive of incipient vesicle formation, were noted. It is appreciated too that, as the lesions were studied at only one point in time, evolution from one type to another will occur in some instances. This is particularly relevant to the erosive lesions which, in the absence of evidence of blister formation in this study, are presumably formed by denudation of epithelium from the pseudo-vesicular or purpuric lesions. Classification of the maculo-erythematous lesion as an oral manifestation of dermatitis herpetiformis was less certain, as this appearance was occasionally noted in control subjects. Nevertheless, the frequent occurrence of these lesions, along with the more definite clinical abnormalities, suggested that they represented a mild manifestation of the disease. The presence of this type alone, however, in patient 3, in the absence of histological confirmation, could not be regarded as firm evidence of oral involvement. As regards the occurrence of isolated purpuric punctae in the mouth in dermatitis herpetiformis, classification as a specific lesion was not acceptable, despite the prevalence of a purpuric element in specific lesions, in view of the similarly high incidence of isolated purpuric punctae in control subjects.

The strikingly non-vesicular and purpuric nature of the oral eruption in dermatitis herpetiformis contrasts with the characteristic skin changes. Nevertheless, in patient 5a, two small pseudo-vesicles occurred on a single erythematous base, thus resembling the familiar vesicular grouping which is the hall mark of the disease on the skin, and the variation in the types of oral lesion, occurring together or at different times, in individual patients could be regarded as consistent with the multiform nature of the skin eruption (Duhring, 1884). Even more striking than the contrast between the appearance of the skin and oral lesions, was the absence of symptoms in oral lesions in all but two patients. The oral mucosa is, however, with the exception of the tongue (on which painful lesions did occur), relatively insensitive.

The distribution of the skin eruption in dermatitis herpetiformis is generally considered to be influenced by local trauma; and so it might be expected that this factor would also influence the distribution of oral lesions. That this indeed occurs is suggested by the fact that lesions were commonly sited either on the mucosa adjacent to the occlusal plane, or under dentures. Indeed, the degree of trauma to which the occlusal plane area is subject is indicated by the frequent occurrence of pin-point haemorrhages and areas of keratinization in this region in the control group. Presumably, trauma is also responsible for the markedly purpuric nature of the oral lesions of dermatitis herpetiformis, and it is interesting that the other area in which purpuric lesions have been noted in this disease is on the palms and soles (Fraser, Beck & Albert-Recht, 1971; Marks & Wilson Jones, 1971).

Laboratory confirmation of the nature of the oral lesions seen in this study was primarily obtained from histological examination. The significant histological feature of dermatitis herpetiformis affecting the skin is the presence of papillary micro-abscesses, which consist of aggregates of neutrophils and eosinophils at the tips of the dermal papillae (Piérard & Whimster, 1961; MacVicar, Graham & Burgoon, 1963). Similar micro-abscesses can occur at the periphery of bullae in pemphigoid (Bolgert & Chastanet, 1963; Jablonska & Chorzelski, 1963), but their presence in an early non-vesicular lesion may be regarded as diagnostic of dermatitis herpetiformis (Lever, 1967). Papillary micro-abscesses were evident in all nine oral biopsies taken in the present study. Subepithelial vesicle formation was, however, uncommon, presumably due to the tendency for the epithelium over a micro-abscess to split (Fig. 8). This splitting can also be seen in skin biopsies in dermatitis herpetiformis (Piérard &

Whimster, 1961), but may occur more commonly in the mouth in view of the degree of trauma to which the area is subject, and of the absence of a protective keratin layer in the buccal mucosa. Another striking difference from the histology of skin lesions was the frequent presence of large numbers of extravasated red blood cells in the submucosa of the oral lesions. This difference, however, may be simply one of degree, as the presence of small numbers of free red blood cells in the dermis is not uncommon in the skin lesions (Connor, Marks & Wilson Jones, 1972).

Further confirmation of the nature of the oral lesions in dermatitis herpetiformis was obtained with immunofluorescence studies. Examination of involved and uninvolved skin in this disease reveals a characteristic staining pattern consisting of granular deposits of predominantly IgA immunoglobulin at the dermo-epidermal junction (Van der Meer, 1969; Cormane, Szabo & Hauge, 1970; Chorzelski *et al.*, 1971; Cormane & Gianetti, 1971; Holubar, Doralt & Eggerth, 1971). This is most frequently seen in uninvolved skin but can be detected in involved skin, provided early non-vesicular lesions are examined (Chorzelski *et al.*, 1971; Holubar *et al.*, 1971). The granular deposits of IgA occurring in the early mucosal lesions in the present study were similar to the skin IgA deposits referred to above (Fig. 11).

It has generally been accepted, by those who recognize the existence of oral lesions in dermatitis herpetiformis, that the incidence of these lesions is low. Figures quoted range from 1% (Borrie, 1967) to 10% (Graham, 1971); although Schuerman *et al.* (1966) in quoting a figure of 8% from a review of the literature suggested that the real incidence might be much higher. The findings in the present group of patients confirm this prediction, as eleven of the fifteen patients studied exhibited oral involvement; an incidence of over 70%. Indeed, had the four patients with negative oral findings been examined on more than one occasion the incidence might well have been higher, as in three of these patients lesions in the mouth had developed after an oral provocation test with potassium iodide during a previous study (Fraser *et al.*, 1971). This discrepancy between the findings of the present investigation and the accepted incidence in the literature is almost certainly due to a failure to recognize the character of the oral changes in dermatitis herpetiformis; a failure encouraged by the infrequency of symptoms.

From this study, therefore, it may be concluded that oral involvement in dermatitis herpetiformis can by no means be regarded as uncommon, and that the lesions which form, while demonstrating some distinctive clinical and histological features, are clearly part of the same basic morbid process as occurs in the skin.

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Nodular subepidermal fibrosis in non-filarial endemic elephantiasis of the legs

E.W.PRICE

Princess Zenebework Hospital, P.O. Box 5033, Addis Ababa, Ethiopia

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SUMMARY

Nodular subepidermal fibrosis is a common complication of non-filarial elephantiasis of the lower legs. It occurs in the 'rubbery' type of elephantiasis only. The sites of predilection correspond to areas exposed to microtraumata; the sole and the leg above the ankle are never involved by nodulation. The microscopic appearances are described. Treatment, which is demanded by the patient for aesthetic reasons, or required as a preparatory measure for footwear, is surgical removal of the nodules.

Nodular subepidermal fibrosis was defined by Michelson (1933) as a clinical entity in which 'solitary or multiple, hard fibrous sessile tumours are situated subepidermally, arising spontaneously or following slight, but insignificant trauma'. They are distinguished from keloids on the one hand and from true dermal tumours, such as neurofibromas, on the other, and have been called histiocytoma (Lever, 1961; Pinkus & Mehregan, 1969). There is, however, general agreement that the condition is a chronic inflammatory response to minimal trauma (Rentiers & Montgomery 1949).

The present paper draws attention to the frequency of nodular fibrosis in non-filarial endemic elephantiasis of the legs, which is a common disease in Ethiopia, and describes the clinical appearance, pathology and treatment of this condition.

The prevalence is not easy to define, since the thickened skin of elephantiasic feet is often irregular, and small localized swellings may or may not be considered as incipient nodular fibrosis but, at least one example of the condition as illustrated here, can be observed at a clinic seeing 10–15 elephantiasic patients each week.

CLINICAL FEATURES

Nodular fibrosis occurs in one form only of elephantiasis of the lower legs—the so-called 'rubbery' type, in which the thickened oedematous dermis is rubbery to pressure and cannot be picked up between the fingers. In contrast, the oedematous condition in the 'water-bag' type is predominantly subdermal, so that the skin is only moderately thickened, is soft, and easily raised in a fold by the fingers.

The sites and prevalence of nodulation in the rubbery type are presumed to be related to the frequency of microtraumata to the prominent and resistant skin surface, contrasting with the infrequency of damage to the soft yielding surface of the skin in the water-bag type.

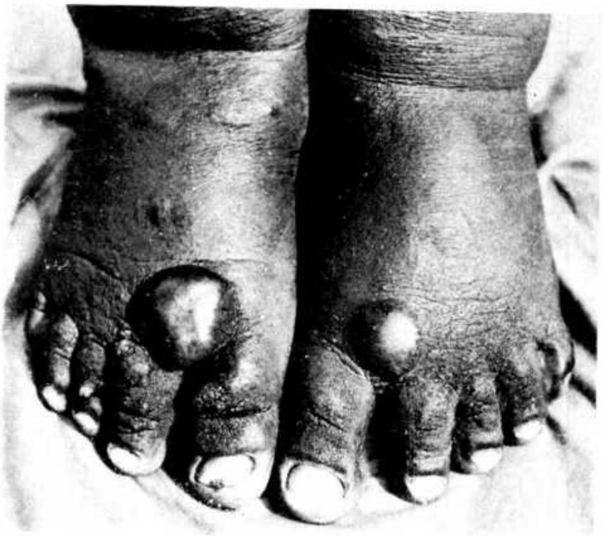

FIGURE 1. Nodular subepidermal fibrosis on elephantiasic feet. The site is determined by counter-irritation applied to the areas of maximum itching at the beginning of the chronic lymphoedema.

Nodules develop symmetrically on the two feet; however, the nodules on one foot are always more developed and larger than on the other, in accordance with the usual asymmetry of the elephantiasic legs. They occur commonly at the base of the medial toe-clefts on the dorsum of the foot. The big-toe cleft is often involved, because of the frequency with which counter-irritation is applied by local traditional practitioners for the persistent itching of incipient lymphoedema in this area (Fig. 1).

Other common sites are along the ridges of the horizontal folds of skin in front of the ankle, which

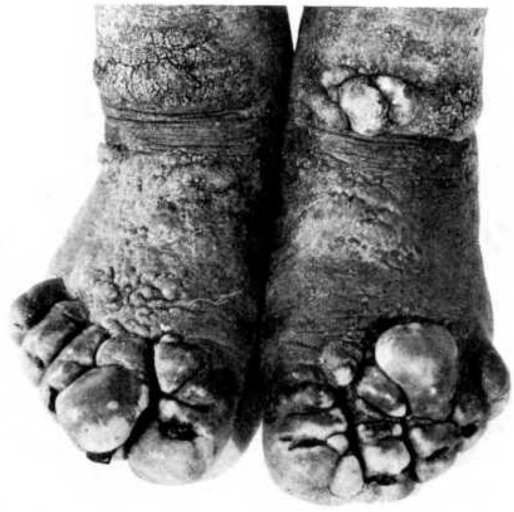

FIGURE 2. Sites of predilection for nodular fibrosis: the dorsum of toes and foot, the base of the toe-clefts, and the horizontal ridges of the ankle folds. The lesions are symmetrically placed, but one side is always more advanced than the other.

result from repeated dorsiflexion of the elephantiasic tissues (Fig. 2). They are also common on the dorsa of the toes, but not on the tips, plantar or lateral surfaces. They are never seen on the sole, nor above ankle level. When they are numerous, they may occupy large areas, concealing entirely the skin of the dorsum of the foot (Fig. 3).

A nodule begins as a small elevation of the elephantiasic skin; it has not been observed on non-elephantiasic skin. The nodule is circular, sessile, dome-shaped, with a surface which is smooth and shiny, or rough and granular, according as the skin surface under which it developed was smooth or granular. It grows in size from a pin-head to 3 or 4 cm across.

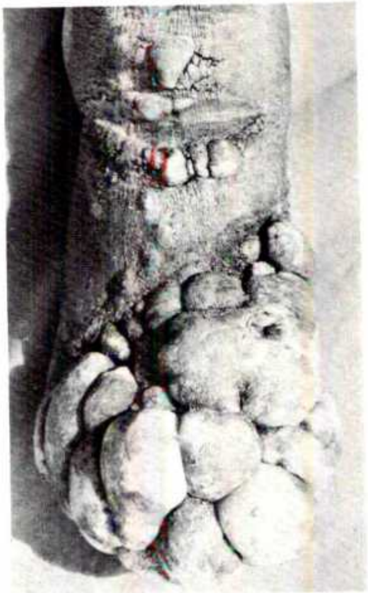

FIGURE 3. Advanced stage of nodulation. When nodules are numerous and crowded, the skin of the dorsum may be completely hidden, but the nodules are then pedunculated, and can always be removed individually. Nodules never appear on the plantar surfaces of toes or foot, nor above the ankle level, no matter how long the elephantiasis has lasted.

The nodule may remain single on each foot and grow in diameter and height, but more commonly multiple nodules develop successively, adjacent to each other. When the nodules are numerous and crowded together, they tend to become pedunculated. The spaces between the nodules are often secondarily infected, and exudation produces an odour which reduces the patient to a miserable condition.

The nodules are rigid and hard, so that the tops are exposed to trauma, infection and ulceration followed by hypopigmented scarring. Malignant change of chronic ulceration has not been observed.

The developed nodule is insensitive to pain, in contrast with the adjacent elephantiasic skin. The mobility on underlying tissues, which is a feature of nodular fibrosis in non-elephantiasic patients, cannot be demonstrated in these cases, since the skin is itself adherent to underlying tissues.

PATHOLOGY

Macroscopically (Fig. 4) a nodule is a dense, hard, dome-shaped structure, the removal of which may

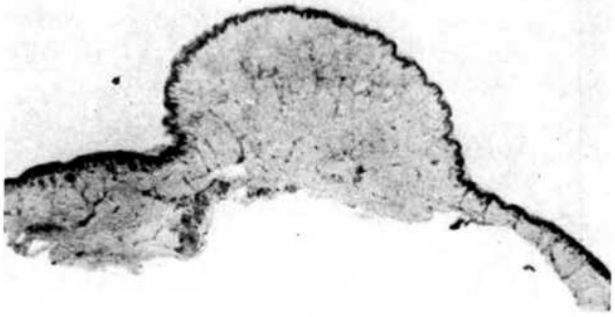

FIGURE 4. In this photomicrograph of a nodule, the lymphocytic cuffing of the sweat ducts just below the epidermis can be seen in the elephantiasic skin. It will be observed that this is absent over the nodule (H & E, × 12).

break the scalpel blade. Its limits cannot be demarcated from the adjacent and underlying fibrotic dermis on which it lies, but it is superficial to the level of the sweat glands. The epidermis is loosely attached to the nodular substance and readily detaches on traction with forceps. On removal of the nodule, a single blood supply is found at the base. The incised surface is uniformly white, with minute pin-point haemorrhages from the scattered blood vessels. No hairs are present over the nodule.

The characteristic microscopic changes are limited to the volume superficial to the level of the sweat glands. These glands, which are embedded in the ordinary elephantiasic tissue, continue across the base of the nodule to its far side, but are then often atrophic, with elongated and distorted ducts leading through the nodule to the surface. Contrary to the common finding in elephantiasic skin, these sweat ducts do not have a subepidermal lymphocytic cuff or collar.

No pilosebaceous apparatus is present.

The bulk of tissue consists of bundles of collagen fibres in irregular whorls, often proceeding into the papillary ridges (Fig. 5), but some areas may show an interval of normal subepidermal connective tissue, as described by Lever (1961). The nuclei may be stellate or finely rod-shaped, few or numerous, according to the age of the tissue, but in all cases, the collagen stained pink with eosin. On axon staining, no nerve fibres could be demonstrated. Blood vessels were few, and were sometimes surrounded by collections of small round cells.

TREATMENT

Treatment is demanded by the patient either for aesthetic reasons, or is advised in preparation for the provision of footwear as treatment for elephantiasis.

When there are few nodules, removal is simple, but even multiple nodules crowded together may be, with patience, removed one by one. The work is greatly facilitated by the insensitivity of the nodules, so that an anaesthetic is not required. This is fortunate since the elephantiasic skin is too dense for infiltration with local anaesthetic. Nodules cannot be removed totally, since the edges of elephantiasic skin will not then approximate for suture. A wedge-shaped incision crosses the dome, followed by formation of flaps of tissue sufficient to close the space left by removal (Fig. 6). A persistent bleeding-point at the base of the nodule will usually need under-suturing. The surgery approximates to a sculpturing of the tough collagenous mass, and suture may not be necessary, nor is it always possible because of the friable edges which remain. Healing follows approximation of the flaps, and

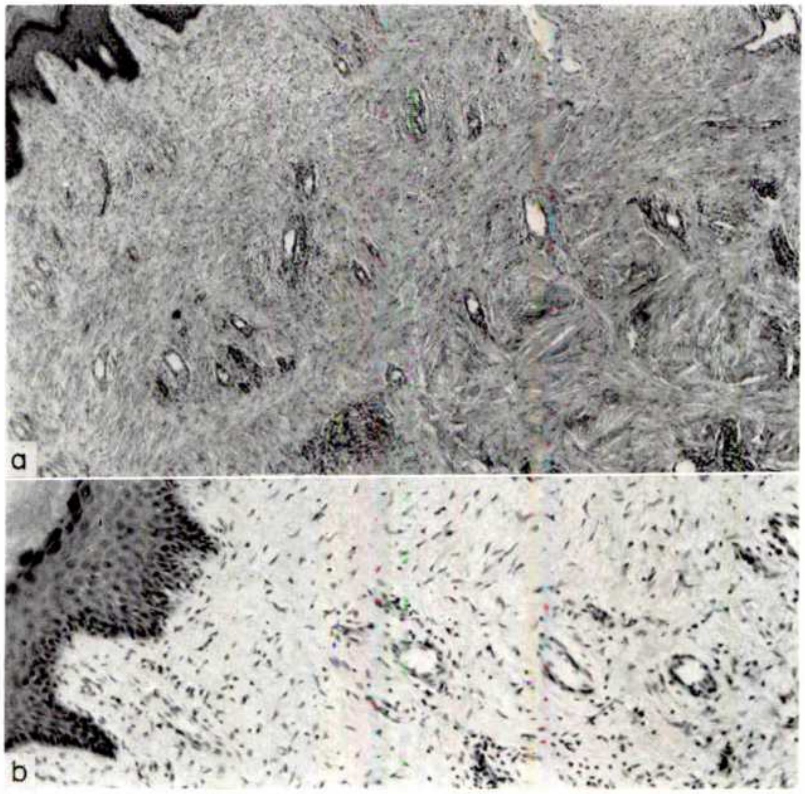

FIGURE 5. Detail of nodule showing irregular whorls of collagenous fibres, with attenuated sweat ducts, a few vessels with small round cell accumulations, and an occasional dilated lymphatic vessel. (a) (H & E, × 70), (b) (H & E, × 140).

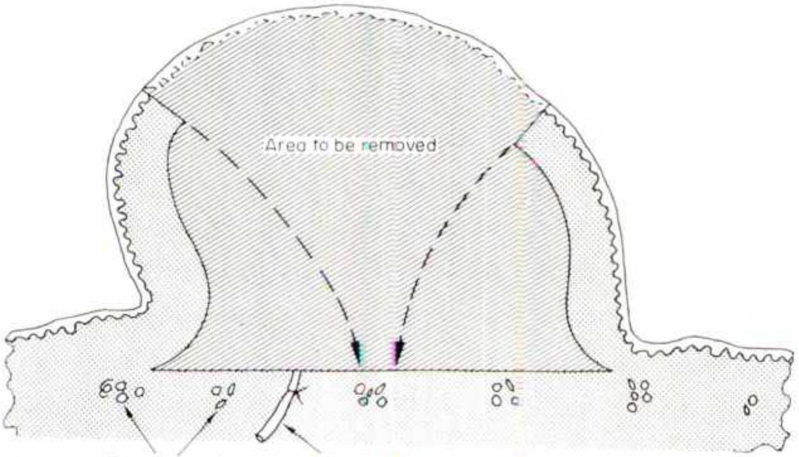

FIGURE 6. Removal of nodule in elephantiasic skin. Two flaps are sculptured as illustrated, to close the gap left by removal of the remainder of the nodule. Ligature of the single blood supply is essential, and may need to be under-run by a threaded needle. No anaesthetic is required, since the nodule is insensitive. Suture of the flaps may not be needed.

the result is aesthetically acceptable and makes possible the provision of footwear. In cases treated in this way as much as 18 months ago, there is no evidence of re-formation of the nodule.

DISCUSSION

The present lesion conforms closely to the nodular fibrosis of Michelson, and appears on areas exposed either intentionally (counter-irritation) or unintentionally, to irritation, as on the summits of the rigid folds of elephantiasic skin. The absence on the sole is related to the density of the epidermal thickening in bare-footed people, preventing access of the irritant to the dermis.

The absence of the lesion above the ankle suggests that the microtraumata, which provoke the lesion, are derived from the ground; and this accords with the hypothesis of a geochemical irritant, discussed elsewhere (Price, 1972; Heather & Price, 1972). The chronic lymphoedema is there attributed to alumino-silicates, or other minerals toxic to tissue, absorbed through microtraumata to the foot.

Nodulation has not been observed in the few cases of elephantiasis of the lower arms.

The area from which the patients come is free from *Wuchereria bancrofti*.

ACKNOWLEDGMENT

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British Journal of Dermatology (1973) **89**, 457.

Nail anomalies in Hodgkin's disease

R.T.SHAHANI AND E.K.BLACKBURN

Department of Haematology, Weston Park Hospital, Sheffield S10 2SJ, and University Department of Haematology, The Royal Infirmary, Sheffield S6 3DA

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SUMMARY

Peculiar nail anomalies reflecting a poor prognosis are described in four patients with generalized Hodgkin's disease. While all the patients had from one to three complete transverse white lines in each nail, one also showed dark brown coloration of the distal parts of the nails with white proximal parts.

PATIENTS

We were stimulated by recent nail changes in a patient with Hodgkin's disease to screen fifty patients with this disorder. Details relating to the four patients found with nail anomalies are given in Table 1.

TABLE 1.

Case no.	Sex	Clinical stage	Histological Classification	Time to death after appearance of nail changes (months)
1	M	IV B	Lymphocytic predominant	4
2	M	IV B	Mixed cell	3
3	M	IV B	Mixed cell	4
4	F	IV B	Mixed cell	2

The Rye classification (1966) was used for staging the disease. All four patients had one to three transverse white lines in each nail, traversing it from side to side. Each line was 1–2 mm wide and borders were usually smooth. Areas of pink coloration separated the white lines. In addition, Case 1 had dark brown coloration of the distal part of the nails with a white proximal part (Plate 1). The nails were smooth and there was no accompanying dermatological condition such as psoriasis or fungal disease. All changes were more marked in the finger nails than in the toe nails. No relationship between nail anomalies and chemotherapy or radiotherapy was evident, and all the patients had become refractory to specific therapy when the changes appeared.

DISCUSSION

While many nail anomalies have been found in systemic disorders, recently two interesting changes have been reported in acute and chronic renal failure (Lindsay, 1965, 1967; Hudson & Dennis, 1966; Leyden & Wood, 1972). In the first of these, designated 'half-and-half nail', the distal portion of the nail has a dark brown colour, while the proximal part is white. Transverse white lines constitute the second change, which has been termed 'red and white nail' and 'leukonychia striata' by Albright & Wheeler (1964). Muehrcke (1956) observed these lines in patients with chronic hypoalbuminaemia. None of our patients, however, showed clinical signs of renal failure, and the plasma urea and albumin levels were consistently within the normal range.

Transverse white ungual lines were first reported by Sabin (1901) in arsenical poisoning. Since then this change has been noted in a wide variety of systemic disorders, including myocardial infarction, renal failure, leprosy, malaria, pellagra and chronic hypoalbuminaemia. In our review of relevant literature, however, we found only two reports of patients suffering from Hodgkin's disease with transverse white lines of nails. Weber's (1918) patient had ascites, while Ronchese's (1951) patient had a 2-month history of high fever. We have been unable to find any published reference to a combination of 'half-and-half nail' with transverse white lines as occurred in our patient no. 1.

The exact pathogenesis of these nail anomalies is not known. It is believed that transverse white lines are due to pathological keratinization. Wedge biopsies of 'half-and-half nails' (Leyden & Wood, 1972) have shown that there is excessive deposition of melanin in the distal part of the nail, while the proximal part is completely devoid of the pigment.

Although the nail changes in our patients were clearly not specific to Hodgkin's disease, once they were observed they indicated a bad prognosis.

ACKNOWLEDGMENTS

We wish to thank Dr I.B. Sneddon for his advice.

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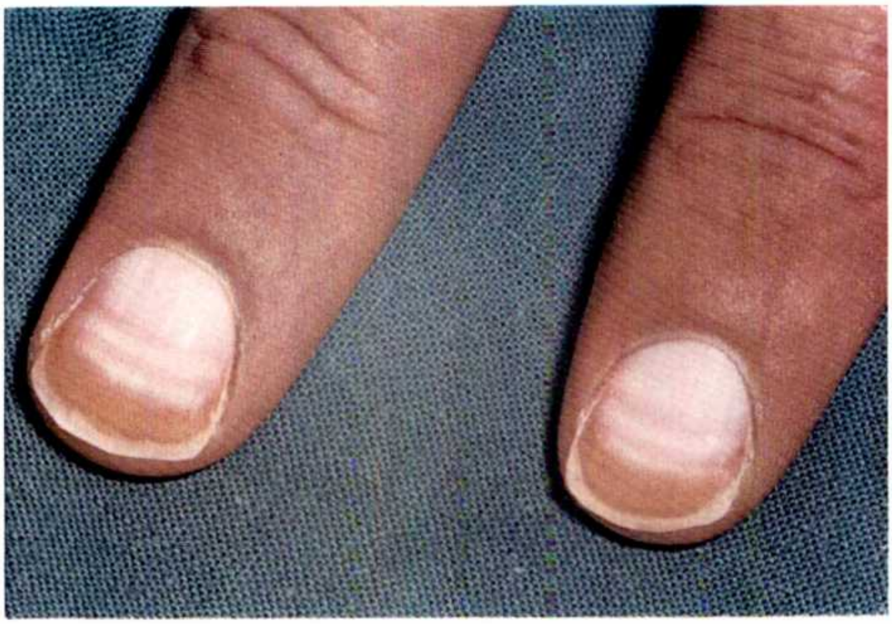

PLATE 1

(facing page 458)

British Journal of Dermatology (1973) **89**, 459.

Hexokinase, glucose-6-phosphate dehydrogenase, and malate dehydrogenase in the isolated sweat glands of normal and atopic subjects

D.W.K.COTTON AND ELISABETH VAN ROSSUM

Department of Dermatology, University of Nijmegen, Nijmegen, The Netherlands

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SUMMARY

The kinetics of the enzymes hexokinase, glucose-6-phosphate dehydrogenase, and malate dehydrogenase have been determined and compared in pooled isolated sweat glands from normal and atopic subjects. No significant differences could be detected in the kinetic constants V_{max} and K_m for any of the substrates or co-factors studied in the two groups of subjects.

The results of these experiments are discussed in relation to the current concepts regarding atopy and atopic dermatitis.

The Szentivanyi (1968) model of the atopic syndrome suggests an endogenous, partial blockade of the β-receptors (adenyl cyclase). We have criticized this model on a number of grounds (Cotton, 1971, 1972), the strongest of which is the demonstration that adenyl cyclase and a number of related enzymes are normal in atopic skin (Mier & Urselmann, 1970; Holla *et al.*, 1972; Mier & van den Hurk, 1972).

There seems good reason to suspect sweat gland involvement in atopic dermatitis on clinical and histological grounds (Mali, 1965) and on pharmacological grounds (Warndorff, 1970; Hemels, 1970). The Szentivanyi model is largely based upon observations regarding carbohydrate metabolism, and it should, therefore, be of interest to compare glucose metabolism in sweat glands from normal and atopic subjects.

This article reports the kinetics of the rate limiting enzymes from the three main pathways of glucose metabolism (glycolysis, hexose monophosphate shunt and Krebs') in isolated sweat glands from normal and atopic subjects.

MATERIALS AND METHODS

None of the patients were receiving topical or systemic therapy at the time of biopsy. Biopsies were taken from normal appearing skin on the upper arm. The skin was first frozen with Chlorethyl spray and a 4 mm diameter biopsy taken with a sharp biopsy punch. The specimens were immediately frozen at −40°C and were stored frozen until sectioned on the cryostat. 16 μm sections were cut and maintained frozen in small tissue-sample holders. These were then placed in evacuation and storage tubes and freeze dried. The freeze dried sections were stored under vacuum at −40°C until used. Fractions were dissected out under a binocular microscope using a Leitz micromanipulator. The dissected fragments were stored under vacuum at −40°C until used. The fractions from several atopic biopsies were pooled, as were those from several normal biopsies. The dried, frozen fragments were homogeneously mixed with a glass rod, and samples were subsequently weighed, suspended in known volumes of buffer, and disrupted by ultrasonication with a Branson 'Sonifier' B.12. The resulting solutions were centrifuged and used directly for the enzyme assays ('skin fraction solutions').

HEXOKINASE

This enzyme catalyses the conversion of glucose to glucose-6-phosphate (G-6-P) according to the reaction:

$$\text{glucose} + \text{ATP} \rightleftharpoons \text{G-6-P} + \text{ADP}$$

This reaction is most readily followed by coupling to glucose-6-phosphate dehydrogenase:

$$\text{G-6-P} + \text{NADP} \rightleftharpoons \text{6-phosphoglucono-}\delta\text{-lactone} + \text{NADPH}$$

and measuring the NADPH produced spectrophotometrically or fluorometrically.

The following solutions were prepared:

(*a*) *Buffer*. Tris 0·05 M + EDTA 5×10^{-4} M + $MgCl_2$ 0·03 M + bovine serum albumin 0·5 g/l, pH 7·2.

(*b*) *Glucose*. 4 mM in (a).

(*c*) *ATP*. 4 mM in (a).

(*d*) *NADP*. 4 mM in (a).

(*e*) *Glucose-6-phosphate dehydrogenase*. 0·1 mg/10 ml in (a).

(*f*) *Hexokinase*. 0·1 mg/10 ml in (a).

(*g*) *Skin fraction solutions*. Approx. 50 μg/ml in (a).

(*h*) *NADPH*. 4 mM in (a).

Various dilutions of (h) were prepared in buffer and the fluorescence measured. The graph of fluorescence versus concentration of NADPH was used to convert the readings from the assays into units of NADPH produced per unit time per unit weight skin extract.

Reaction mixtures consisted of: 0·1 ml (b), 0·1 ml (c), 0·1 ml (d), 0·1 ml (e), 0·1 ml (f) or (g).

The linearity with concentration of enzyme, and the kinetics of the reaction for ATP and glucose were checked with pure enzyme in preliminary experiments.

For variable glucose and ATP concentrations with skin extracts various amounts, up to 0·1 ml of (b) and (c), were used successively, and the volume made up to 0·5 ml with buffer (a).

The mixtures were placed in a quartz microcuvette and the change in fluorescence (excitation 350 nm, emission 450 nm) was followed. The fluorimeter was a Baird Atomic Spectrophotofluorimeter SF 100 fitted with a thermostatted microcuvette holder. All measurements were done at 37°C. The controls on the fluorimeter and the recorder were adjusted to give initial velocities of approximately 45° on the recorder paper.

Results

Lineweaver Burk plots for ATP and glucose with the sweat gland extracts from both normal and atopic groups are shown in Figs. 1 and 2 respectively.

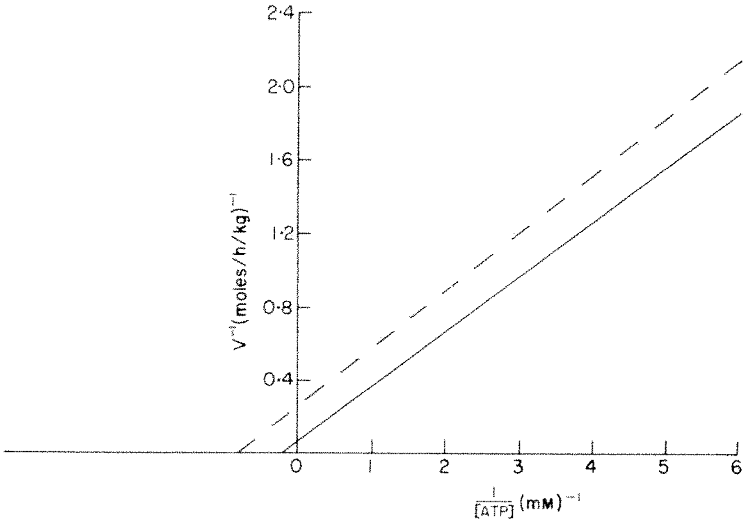

FIGURE 1. Lineweaver and Burk plots of hexokinase activity from sweat glands with variable ATP concentration. Solid line normals, broken line atopics. Each line is the mean plotted through duplicate experimental points.

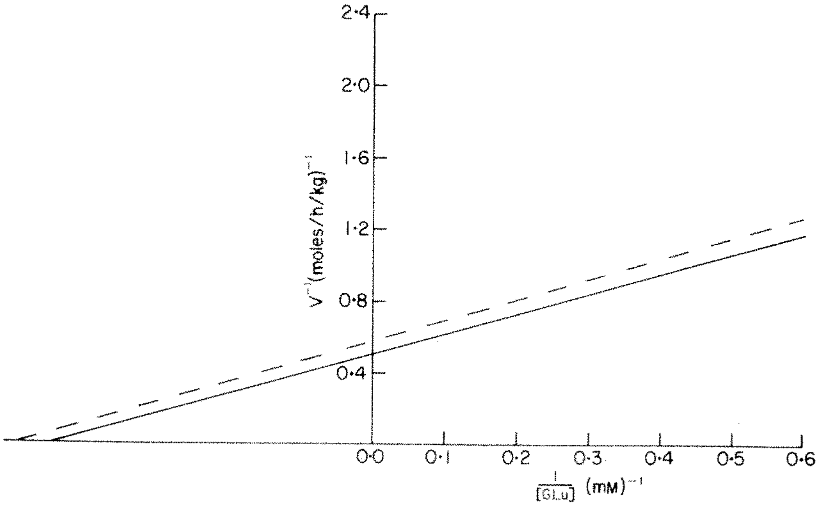

FIGURE 2. Lineweaver and Burk plots of hexokinase activity from sweat glands with variable glucose concentration. Solid line normals, broken line atopics. Each line is the mean plotted through duplicate experimental points.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE

This enzyme catalyses the conversion of glucose-6-phosphate to 6-phosphoglucono-δ-lactone according to the reaction:

$$\text{G-6-P} + \text{NADP} \rightleftharpoons \text{6-phosphoglucono-}\delta\text{-lactone} + \text{NADPH}$$

The reaction can be followed spectrophotometrically of fluorometrically by absorbence or intrinsic fluorescence of NADPH.

The following solutions were prepared:

(*a*) *Buffer*. 0·05 M+EDTA 5×10^{-4} M+$MgCl_2$ 0·03 M+bovine serum albumin 0·5 g/l, pH 7·2.

(*b*) *G-6-P* (disodium salt). 14 mM in (a).

(*c*) *NADP*. 4 mM in (a).

(*d*) *G-6-P dehydrogenase*. 0·1 mg/10 ml in (a).

(*e*) *Skin fraction solutions*. Approx. 50 μg/ml in (a).

Since the end product measured in this assay is the same as in the previous (hexokinase) assay the same conversion graph for NADPH/fluorescence was used.

Reaction mixtures consisted of: 0·1 ml (b), 0·1 ml (c), 0·2 ml (a), 0·1 ml (d) or (e).

The linearity of the reaction with concentration of enzyme and the kinetics for G-6-P and NADP were checked with pure enzyme in preliminary experiments. For variable G-6-P and NADP concentrations with skin extracts variable amounts, up to 0·1 ml, of (b) and (c), were used successively, and the volume made up to 0·5 ml with buffer (a).

The reagents were placed in a quartz microcuvette and a base line was recorded without G-6-P present. The reaction was started with G-6-P and the change in fluorescence (excitation 350 nm, emission 450 nm) was followed at 37°C, as for hexokinase.

Results

Lineweaver and Burk plots for G-6-P and NADP with the sweat gland extracts from both normal and atopic groups are shown in Figs. 3 and 4 respectively.

MALIC DEHYDROGENASE

This enzyme catalyses the conversion of malic acid to oxaloacetate according to the reaction:

$$\text{L-malic acid} + \text{NAD} \rightleftharpoons \text{oxaloacetate} + \text{NADH}$$

However, the equilibrium is far to the left with an equilibrium constant:

$$(\text{oxaloacetate} \times \text{NADH} \times \text{H})/(\text{L-malate} \times \text{NAD}) = 6{\cdot}4 \times 10^{-13}\ \text{M}$$

Therefore, under normal conditions it is almost impossible to assay the enzyme using the forward reaction. The reverse reaction can be studied or the equilibrium can be shifted to the right with a

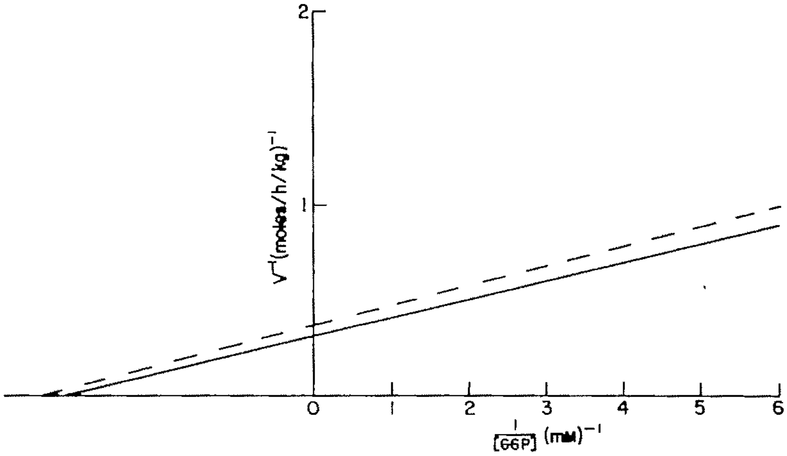

FIGURE 3. Lineweaver and Burk plots of G-6-P dehydrogenase activity from sweat glands with variable G-6-P concentrations. Solid line normals, broken line atopics. Each line is the mean plotted through duplicate experimental points.

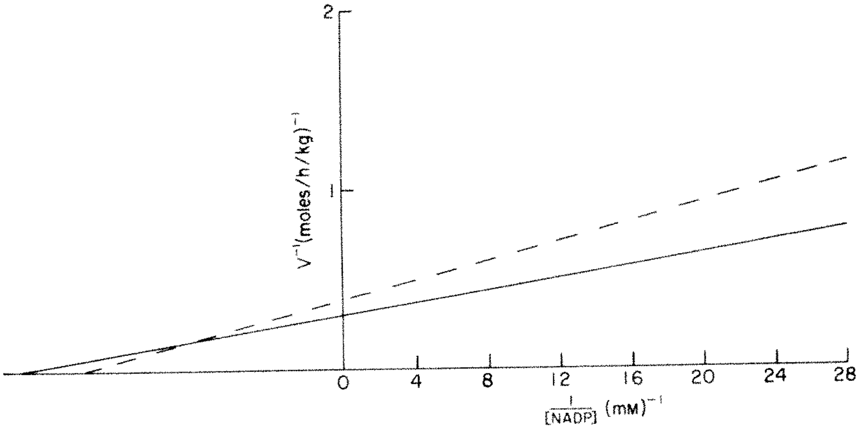

FIGURE 4. Lineweaver and Burk plots of G-6-P dehydrogenase activity from sweat glands with variable NADP concentration. Solid line normals, broken line atopics. Each line is the mean plotted through duplicate experimental points.

high pH and a system of removing the oxaloacetate. The assay used here is based on the latter technique. In this case the reaction can be followed continuously via the NADH produced.

The following solutions were prepared fresh each day:

(*a*) *Buffer.* Hydrazine hydrate 0·4 M + glycine 0·5 M, pH 9·5, + bovine serum albumin 0·5 g/l.
(*b*) *Malic acid.* 1·56 mM in (a).
(*c*) *NAD.* 2·42 mM in (a).
(*d*) *Malate dehydrogenase.* 0·1 mg/10 ml in (a).
(*e*) *Skin fraction extracts.* Approx. 50 μg/ml in (a).

A conversion graph was prepared for NADH in hydrazine buffer (a).

Reaction mixtures consisted of: 0·1 ml (b), 0·1 ml (c), 0·2 ml (a), 0·1 ml (d) or (e).

The linearity of the reaction with concentration of enzyme and the kinetics for malate and NAD were checked with pure enzyme in preliminary experiments. For variable malate and NAD concentrations with skin extracts various amounts, up to 0·1 ml of (b) and (c), were used successively, and the volume made up to 0·5 ml with buffer (a).

The reagents were placed in a quartz microcuvette and a base line was recorded without malate present. The reaction was started with malate and the change in fluorescence was followed as for hexokinase.

Results

Lineweaver and Burk plots for L-malate and NAD with the sweat gland extract from both normal and atopic groups are shown in Figs 5 and 6 respectively.

DISCUSSION

The results reported here show no difference between the kinetic parameters of the enzymes studied in the sweat glands of the two groups. The normal variation in enzyme kinetics that can be expected in skin from different subjects is approximately 2 : 1, and might be expected to be considerably less for pooled isolated adnexa; our results for V_{max} values are well within this range. The experimental error in the techniques being ±5% on duplicate assays, we might also expect K_m variations within a

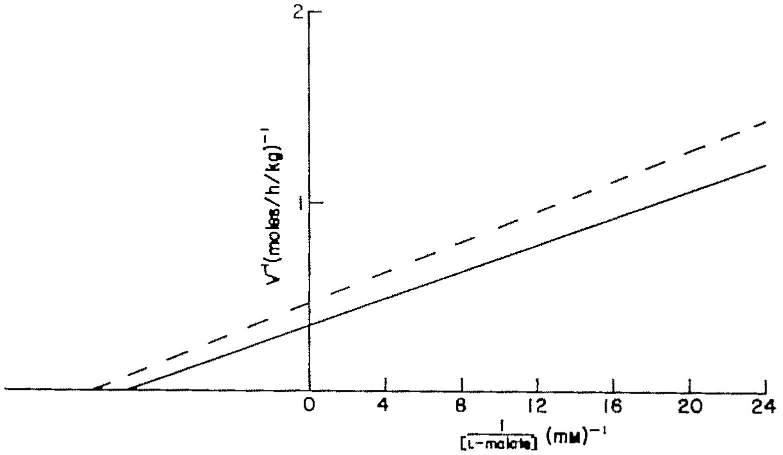

FIGURE 5. Lineweaver and Burk plots of malate dehydrogenase activity from sweat glands with variable L-malate concentration. Solid line normals, broken line atopics. Each line is the mean plotted through duplicate experimental points.

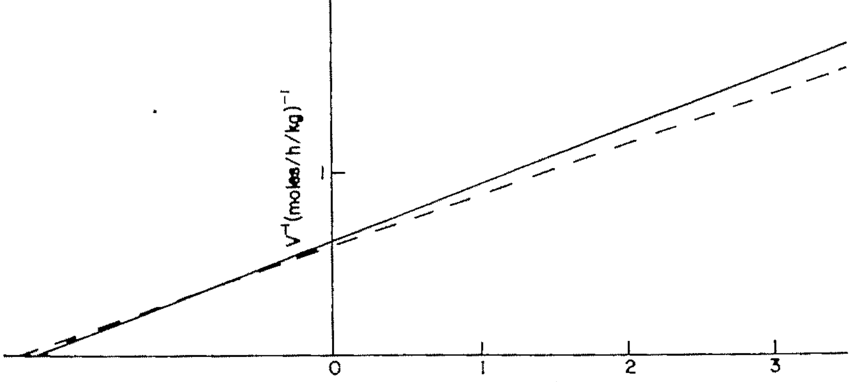

FIGURE 6. Lineweaver and Burk plots of malate dehydrogenase activity from sweat glands with variable NAD concentration. Solid line normals, broken line atopics. Each line is the mean plotted through duplicate experimental points.

3:1 range (Cotton & Mier, 1972). Our values for atopics and normals are well within these limits. Thus, in terms of the enzymes studied, the atopic sweat gland seems to be normal.

The initial concept of atopy as a purely immunological disorder (Coca & Cooke, 1923) has been strongly supported by a number of authors (Frick, 1966), but is now generally not accepted, at least for atopic dermatitis (Champion & Parish, 1968). The more generally acceptable concept is of an imbalance within the autonomic nervous system. The most refined version of this argument has been presented by Szentivanyi (1968), but as we have pointed out previously (Cotton 1971), it is very difficult to specify the point in a cybernetic system that is at fault if the only evidence is an overall disturbance. Recent research, prompted by the Szentivanyi model, has been directed to local abnormalities in the skin (Mier & Urselmann, 1970; Holla *et al.*, 1972; Mier & van den Hurk 1972; Warndorff, 1970; Hemels, 1970; Cotton, Sutorius & Urselmann, 1971; Cotton & van den Hurk, 1972;

Cotton, van den Hurk & Mier, 1973a and b); so far all of these studies have yielded negative results. The work reported here is also negative. This means that, at least as far as sweat gland is concerned, the skin of the atopic is indistinguishable from that of the non-atopic, according to all of the enzymatic parameters so far studied. The reaction pattern to stress and to various pharmacological agents is, however, distinguishable from that of the non-atopic. The possibility that the abnormality in atopy lies in some central or more generalized mechanism, distant from the site of the symptoms, is now very strong, and further research into the psychology (Kuypers, 1968; Kuypers & Cotton, 1972) and the hypothalamic physiology (Benzinger, 1969; Szentivanyi, 1968) of these subjects seems to be indicated.

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The thickness of the epidermis

JUDI T. WHITTON AND J. D. EVERALL

Central Electricity Generating Board, Health Physics Section,
Berkeley Nuclear Laboratories, Berkeley, Gloucestershire, and
Skin Department, The Royal Marsden Hospital, Fulham Road, London, S.W.3

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SUMMARY

Epidermal thickness has been accurately measured on 214 samples of skin from 188 volunteers. Variations with the individual, body site, sex and age are described and discussed.

An original technique of measurement has been developed which has several advantages over conventional methods. In particular, quick straight-forward measurements are possible on volunteer skin rather than post mortem material; and the method eliminates errors arising from the skin's elasticity.

These improvements in accuracy have confirmed that much information in the literature on epidermal thickness, is misleading as the results are based on inaccurate techniques of measurement.

Accurate values of the thickness of the epidermis and its variation with body site, age, sex and so on are important in the fields of medical and biological research.

The variations in skin reaction to certain stimuli could be due to biological differences such as the epidermal thickness, and it may be possible to correlate these variations with the dependence of epidermal thickness on the body site, sex and age.

Knowledge of the physical dimensions of the skin is also important in skin grafting where the success of a graft depends to some extent on matching the skin from the donor and recipient sites.

Certain studies concerned with the effects of ionizing radiations of low penetrating power, such as beta- and alpha-radiation, also utilize knowledge of the physical dimensions of the skin in order to estimate or measure doses to different parts of the tissue. This is particularly important in the nuclear power industry where the dose to the basal layer of the skin (the deepest epidermal layer) can set the limit on the time operators are allowed to perform certain work. Hence, it is essential to have realistic values of epidermal thickness in order that measurements can be interpreted in terms of the dose to the basal layer. False values of epidermal thickness on the sites of interest may result in workers either receiving an unacceptably high skin dose, or on the other hand unnecessary restrictions may be imposed at a serious cost to the industry.

It is surprising, therefore, that there is very little information on the physical dimensions of skin available in the literature. The results of measurements carried out in the latter part of the nineteenth century (Krause, 1876; Drosoff, 1879) are very well established (Leider & Bunke, 1954; Wray, 1963) in present day literature. A detailed comparison of different authors' methods and results (Whitton, 1971) has shown, however, that many of the accepted values, including that of 7 mg/cm^2* suggested by the International Commission on Radiological Protection (1959) for minimal epidermal depth, are highly suspect, as corrections are rarely given for the shrinkage of skin after excision. Also most of the available measurements are from post mortem rather than volunteer skin.

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* 1 $mg/cm^2 \approx 10\ \mu m$ in tissue.

The work described in this paper was initiated primarily to determine accurate values of epidermal thickness which can be used in the nuclear power industry to calculate the radiation dose to the skin. As it appeared, however, that much original information was to be gained from the data, the measurements were extended to give a more comprehensive analysis of epidermal thickness, and we have presented and discussed the results very generally. A more detailed analysis of the importance of these results in radiological protection will be given elsewhere (Whitton, 1973).

MEASUREMENT TECHNIQUE

In order to counteract the natural shrinkage of skin, Southwood (1953) devised a method in which post mortem skin was first marked with a template then restretched to its original size after excision, and pinned down. The skin was then fixed, stained and sliced by standard techniques and the depth of the basal layer below the skin surface measured microscopically at several places along the sample. This technique cannot conveniently be applied to living subjects since a large area of skin is required.

In our measurements, smaller samples of skin were taken from volunteers using a 3 mm diameter punch (Fig. 1), and immediately immersed in sodium bromide solution. This is a standard technique to separate the epidermis from the dermis.

After immersion the epidermis was easily peeled off, washed, dried in a dessicator for reproduci-

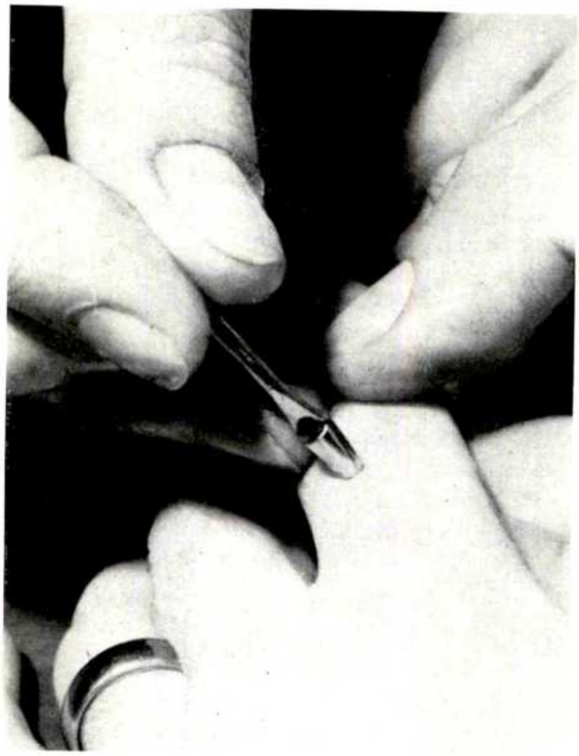

FIGURE 1. Removal of skin sample.

bility and weighed. Checks were made to ensure that no dermis was attached to the peeled off epidermis and that no epidermis was left behind on the dermis. Care was taken also to choose samples where large hair follicles did not interfere with the technique.

The relationship between the dried biopsy weight and thickness measured using Southwood's technique was determined by a series of measurements on post mortem skin. The average epidermal thickness of a fairly large piece of skin was correlated with the average weight of the epidermis from a number of adjacent punch biopsies. A check was made to ensure that any distortion of the skin due to removing the Southwood sample did not affect the biopsy. It was assumed that any physiological change in post mortem skin would affect both measurements equally.

The factor relating weight and thickness was not greatly influenced by the absolute epidermal thickness, although a slightly different correlation factor was used on the samples having comparatively thin epidermis than on those with a thicker epidermis.

As the basal layer undulates with respect to the skin surface each value of epidermal thickness given in this paper is the average for a particular sample. The degree of undulation at different body sites was measured on some of the Southwood samples.

The biopsies were removed from volunteers attending an Out-patient clinic at the Royal Marsden Hospital, London, and from the scientific staff at Berkeley Nuclear Laboratories, Gloucestershire. No anaesthetic was used and care was taken to ensure that the biopsy was cut perpendicular to the skin surface. To prevent distortion of the skin a very sharp instrument was used and little pressure was exerted during the procedure.

Most body sites were sampled. 214 samples were removed from 188 people. Forty-four samples were from female volunteers and the total age range studied was 15–89 years. Volunteers were all Caucasian and had a large cross-section of occupations, about 40% being currently employed in some sort of manual work.

RESULTS

The ratio of the dried biopsy weight to the true epidermal thickness for biopsies of weight $< 350\ \mu g$ was $100\ \mu g \equiv 33{\cdot}3\ \mu m$, and for biopsies of weight $> 350\ \mu g$ was $100\ \mu g \equiv 24{\cdot}4\ \mu m$. These correlation factors were obtained using fifty-seven post mortem samples from nineteen cadavers.

Initial inspection of the results of the main experiment indicated that the most pronounced variations of epidermal thickness occurred between different body sites. The results for twenty-two individual body sites and various groupings of sites are shown in Table 1. The minimum, mean and modal epidermal thickness for each body site are given together with the standard deviation.

The results were finally grouped into six main body site regions and these are emphasized in Table 1 and shown graphically in Figs. 2–5.

Variations of epidermal thickness with sex and age were observed within the six main body site regions. These are shown in Figs. 4 and 5 where the epidermal thickness for all the samples lying within the same 10-year range (0–10 years, 11–20 years, and so on) are averaged.

The results for males and females are shown separately, except in Fig. 5c where the values given are only for the males. This is because these measurements have been further divided into three separate regions on the fingers, fingertips, sides of fingers and backs of fingers. Figs. 4 and 5 also show the number of samples available within each 10-year age interval.

DISCUSSION

Elasticity of skin and comparison of our results with those of other authors

Skin is inherently elastic and when a piece is removed from the body it quite noticeably distorts

TABLE 1. Minimum, modal and mean epidermal thickness together with standard deviation for twenty-two individual body sites, six main body sites and various other body site groupings

Body site no.	Body site	No. of samples (n)	Minimum epidermal thickness (µm)	Modal epidermal thickness (µm)	Mean epidermal thickness (µm)	Standard deviation (µm)
1	Forehead	17	21		50·3	20·2
2	Temple	10	17		52·8	27·5
3	Cheek	3	32		38·8	9·7
	Face (1, 2, 3)	30	17	2–4	49·8	21·9
4	Front upper trunk	7	27		37·6	11·1
5	Back upper trunk	24	23		43·4	13·4
	Upper trunk (4, 5)	31	23		42·2	13·0
6	Front lower trunk	3	35		46·6	10·8
7	Back lower trunk	2	42		43·2	—
	Lower trunk (6, 7)	5	35		45·2	7·9
	Front trunk (4, 6)	10	27		40·0	11·3
	Back trunk (5, 7)	26	23		43·4	12·8
	Trunk (4, 5, 6, 7)	36	23	4–6	42·4	12·4
8	Upper arm	7	37		53·4	25·9
9	Forearm	20	36		60·9	20·0
	Arm (8, 9)	27	36		59·0	18·8
10	Thigh	11	27		54·3	17·5
11	Lower leg and ankle	7	49		74·9	14·5
	Leg (10, 11)	18	27		61·8	18·9
	Arms and legs (8, 9, 10, 11)	45	27	4–6	60·1	18·7
12	Back of hand (radial aspect)	10	50		72·0	12·0
13	Back of hand (ulnar aspect)	19	49		95·3	24·5
14	Back of hand (centre)	4	38		67·0	18·2
	Back of hand (12, 13, 14)	33	38	8–10	84·5	26·2
15	Back of wrist (ulnar aspect)	13	57		87·8	20·3
16	Back of wrist (radial aspect)	11	42		81·6	17·1
	Back of wrist (15, 16)	24	42		85·0	19·3
	Back of wrist and hand (12, 13, 14, 15, 16)	57	38		85·3	23·3
17	Front of wrist (ulnar aspect)	10	45		70·0	17·7
18	Front of wrist (radial aspect)	3	63		73·4	15·3
19	Side of wrist (ulnar aspect)	2	80		95·4	—
	Front and side of wrist (17, 18, 19)	15	45		74·0	14·6
	Wrist (15, 16, 17, 18, 19)	39	42	8–10	80·7	18·4
	Back of hand and front and back of wrist (12, 13, 14, 15, 16, 17, 18, 19)	72	38		82·5	22·4
20	Fingertip	9	176	(40–48)	369·0	111·9
21	Side of finger	7	97		222·7	92·9
22	Back of finger	15	45	(16–20)	138·1	47·3
	Finger (20, 21, 22)	31	45	16–24	221·8	122·0
	Back of hand, wrist and finger (12, 13, 14, 15, 16, 22)	72	38		96·3	38·7

(Evans, Cowdry & Nielson, 1943). The degree of shrinkage depends on both the natural elasticity of the skin, being less in an older person than a younger person, and on the direction of the dermal elastic fibres in the excised sample (Cox, 1941). Evans *et al.* (1943) demonstrated that the surface area of a sample from a young adult decreased by about a factor of two after excision. This is in agreement with our observation that accurate measurements of epidermal thickness are on average about half those recorded by authors who neglected shrinkage (Whitton, 1973).

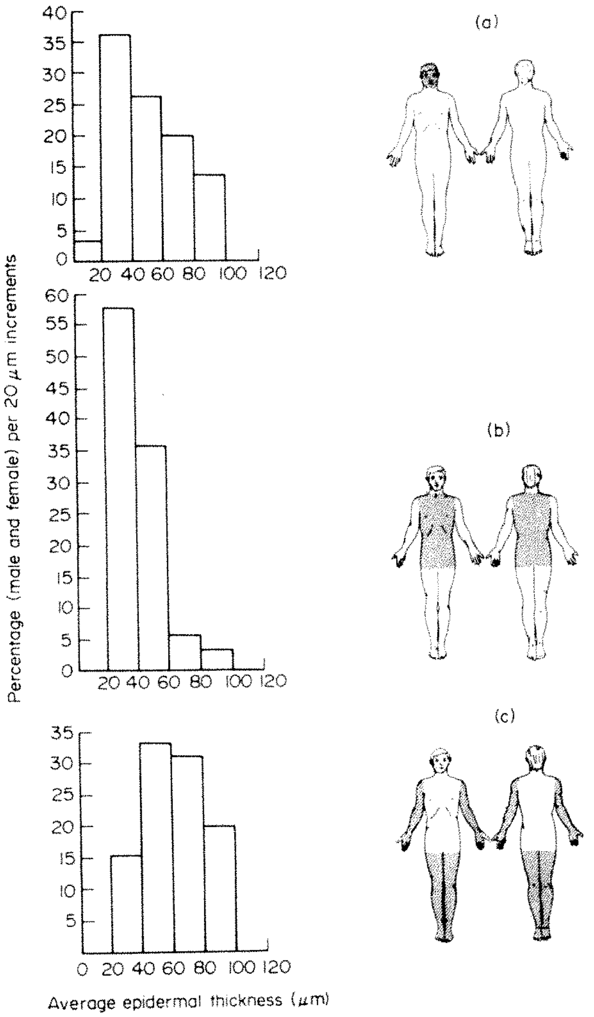

FIGURE 2. Distribution of average epidermal thickness on three regions of the body. (a) face, (b) trunk, (c) arms and legs.

Variation of epidermal thickness with body site

Values of epidermal thickness from 17 μm on the face to 485 μm on the fingertip have been recorded. The thickness of the epidermis appears to be very closely related to the body site. Student's '*t*' test was used to compare the mean epidermal thicknesses on the six main body site regions. At the 5% level of significance the face and trunk were shown to have the same thickness, and the wrists and backs of hands were also the same. Otherwise each of the six sites was significantly different from the others.

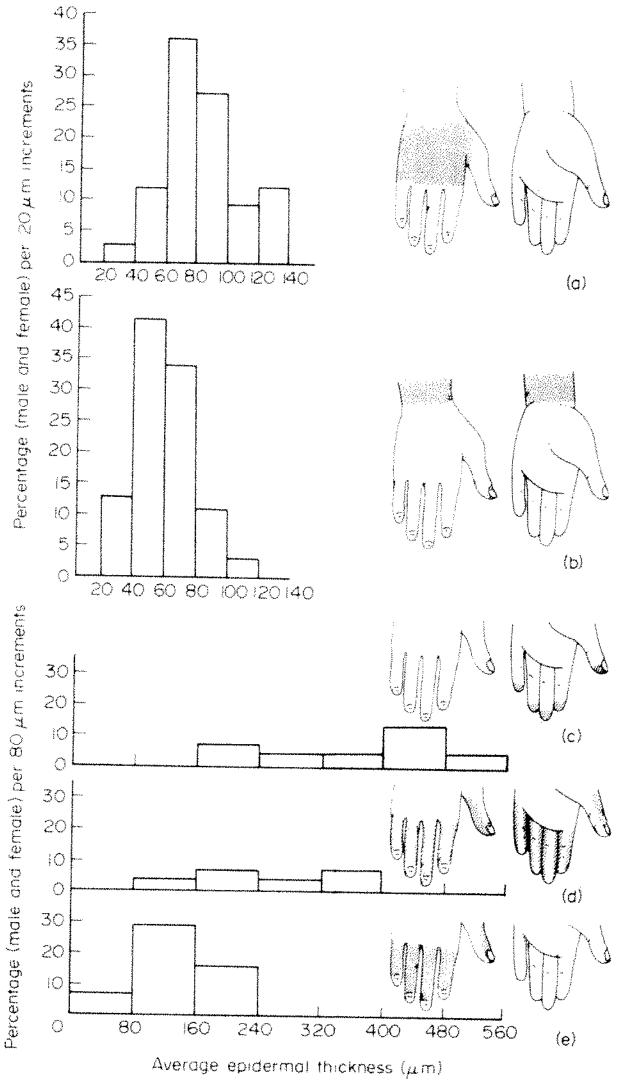

FIGURE 3. Distribution of average epidermal thickness on five regions of the hands and wrists. (a) backs of hands, (b) wrists, (c) fingertips, (d) sides of fingers, (e) backs of fingers.

As the dorsal and ventral surfaces on most parts of the body have a different appearance, a comparison was also made between epidermal thickness on these sites. Although there were insufficient measurements to compare the backs and fronts of the arms and legs, Student's '*t*' test did show that there was no significant difference between the ten readings on the front trunk and twenty-six readings on the back trunk, and between thirteen readings on the front of the wrist and twenty-four readings on the back of the wrist. There was a difference, however, between the front and back of the hands and fingers.

When Student's '*t*' test was used to compare ten readings on the radial side of the back of the hand with ninteen readings on the ulnar side, it was found that the means were significantly different just at the 5% level.

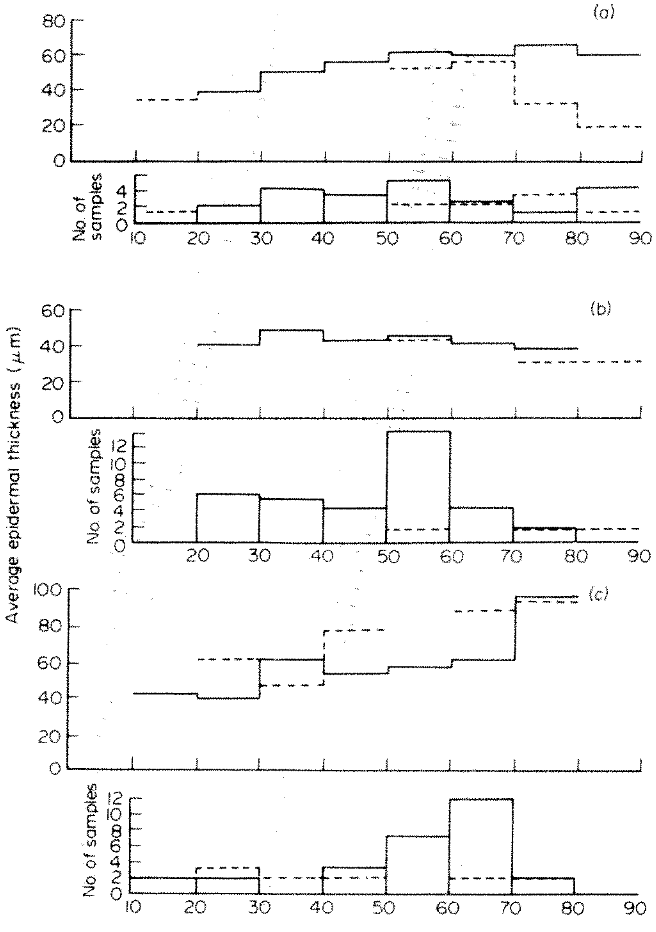

FIGURE 4. Variations of epidermal thickness with age on three regions of the body. (a) face, (b) trunk, (c) arms and legs. ———, male; – – – –, female.

Although we have not attempted to correlate epidermal thickness on the different body sites with the distribution of various skin diseases, the data in this report would provide the basis for an interesting comparison.

Excellent data on the incidence of skin cancer on various body sites have been given by Brodkin, Kopf & Andrade (1969).

Variation of epidermal thickness between individuals

It is possible that the distribution of epidermal thickness as shown in Figs. 2 and 3 is affected by experimental errors, variation between individuals and variation between individual body sites within the main body site grouping. The trunk (Fig. 2b), having the least variation of thickness, shows the limit of the likely experimental error for the other body site, assuming this error is the same for each sample. Before grouping the results a preliminary check was made on whether there was any obvious difference between specific body sites within one of the main body site groups, such as comparison between the front and back of the wrists. Although there were very few samples from some areas there was no outstanding difference, except for the 'finger', which has been further subdivided in Fig. 3c. It seems likely, therefore, that the variations of epidermal thickness shown in Figs. 2 and 3 are primarily influenced by the variation between individuals.

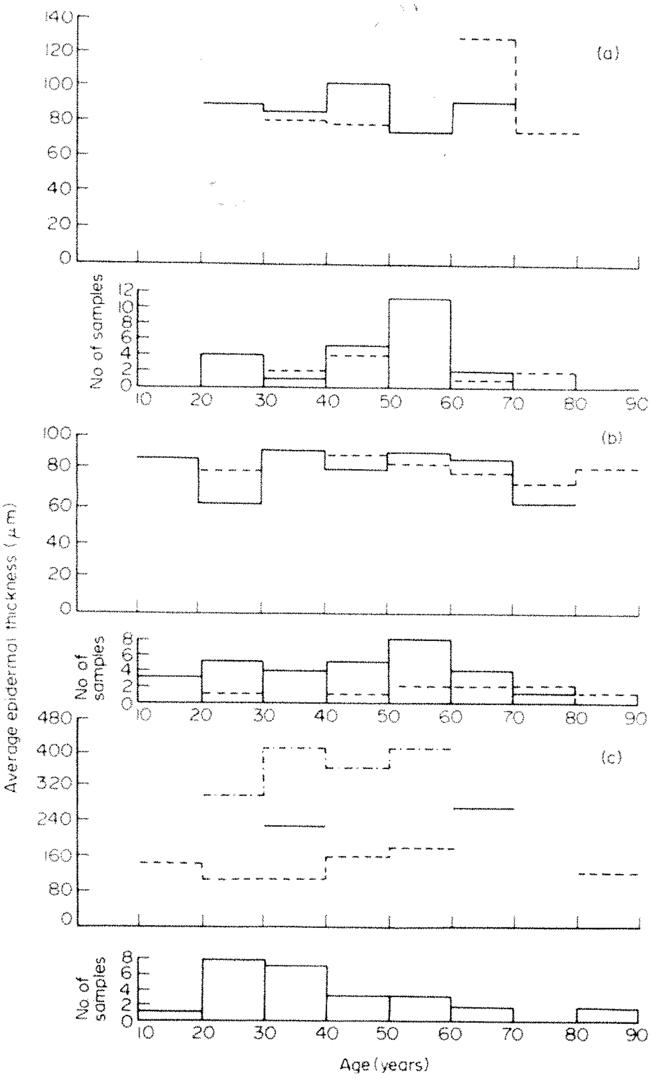

FIGURE 5. Variation of epidermal thickness with age on five regions of the hands and wrists. (a) backs of hands, (b) wrists. ———, male; – – – –, female. (c) fingers, male only. –·–·–·, fingertips; ————, sides of fingers; – – – –, backs of fingers.

It is interesting to observe that the degree of variation on the normally clothed body sites, such as the trunk, is less than that on the normally unclothed sites. This suggests that epidermal thickness is affected to some extent by environmental factors, and supports the observations of Freeman *et al.* (1962) that sunlight affects the physical dimensions of the skin.

In addition to the overall difference in range of epidermal thickness on the various body sites there is a difference in the distribution of thickness about the mode. For example, on the backs of the hands and wrists there are as many samples thinner than the mode as there are thicker, whereas for the arms, legs and face there are more thicker samples and for the trunk more samples have a thinner epidermis. The significance of this is not understood at the present time and more data on individual body sites would be useful.

Tosti, Scerrato & Fazzini (1959) carefully measured epidermal volume on forty-three biopsy samples, which included normal as well as hyperacanthotic and atrophic epidermis. They were primarily interested in the relationships between the number, form and size of the epidermal cells, rather than the absolute determination of epidermal thickness. However, they did conclude that tissue volume may be related to functional phases of the epidermal turnover rather than be a permanent property of a given epidermis. It is not expected that this would grossly influence the conclusions of our work, but it would be interesting in any future studies to relate Tosti's work and our results.

The variation of epidermal thickness with age

The variation of epidermal thickness with age, shown in Figs. 4 and 5, should be considered in conjunction with the subsidiary graphs showing the number of samples measured in each 10-year interval. Too much stress should not be given to those values derived from only one or two samples, such as the measurement from the backs of the hands in the female aged between 60 and 70 years (Fig. 5a).

Despite these irregularities there is a remarkable uniformity of epidermal thickness with increasing age on each of the body sites. Only on the arms and legs does there appear to be a consistent trend of increasing epidermal thickness with advancing age (Fig. 4c) and again the measurements from the volunteers between 70 and 80 years do not carry much weight.

It is a commonly accepted belief that the epidermis is thinner in an elderly person than a younger person. This may have arisen from the fact that the skin looks thinner in the elderly and that measurement of the skin which fails to correct for shrinkage would show an apparent thinning of the epidermis with advancing age. This arises because skin becomes less elastic with age and the errors in the thickness measurements become less.

The variation of epidermal thickness with sex

Although there are comparatively few readings from females compared with males there appears to be no consistent difference between the sexes. One exception may be on the face (Fig. 4a), however, where the average epidermal thickness for the eight females aged over 50 years is about 40 μm compared with that of 55 μm for the twelve males aged over 50 years.

The undulations of the basal layer

Observation of the undulations of the basal layer with respect to the skin surface showed that the degree of undulation was related to the average epidermal thickness, although there were several exceptions to this. As a rough rule most of the basal cells appear to lie between $\pm 25\%$ of the average epidermal thickness.

CONCLUSIONS

The thickness of the epidermis measured using conventional techniques can be overestimated by about a factor of two if allowance is not made for the shrinkage of the sample after excision. Many of the values given in the literature are therefore suspect, as corrections are rarely given for the elasticity.

Using a new technique, which automatically compensates for shrinkage and also allows measurements on volunteer skin, we have accurately determined the epidermal thickness on 214 samples from various body sites of 188 people of both sexes and different ages.

It has been confirmed that epidermal thickness varies dramatically with body site. Most of the body surface has an average epidermal thickness of 40–50 μm. This is increased to about 80 μm on the wrists and backs of hands and reaches 400 μm on the front of the fingers.

There appears to be little correlation, however, of epidermal thickness with the sex and age (15–89 years) of volunteers. Hence the difference in the appearance between male and female skin and between the skin of a young adult and that of an older person is not due to a difference in the thickness of the epidermis.

There is evidence to suggest that environmental factors influence the thickness of the epidermis to a small extent.

The degree of undulation of the basal layer with respect to the skin's surface appears to be related to the average epidermal thickness.

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On the relationship between the urea-soluble and citric acid-soluble proteins of bovine snout epidermis

CHRISTOPHER CARRUTHERS

Department of Biochemistry Research, Roswell Park Memorial Institute,
Buffalo, New York 14203, U.S.A.

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SUMMARY

This report presents evidence that the urea-extractable proteins and the citric acid-extractable protein ('prekeratin') of bovine snout epidermis are related.

Prekeratin was solubilized by trypsinization, S-carboxymethylation, 6 mol/l urea or 0·05 mol/l NaOH. Trypsinization or S-carboxymethylation produced at least two species of soluble proteins. 6 mol/l urea at 37°C solubilized prekeratin, but a homogeneous product was not obtained. Depending upon the duration of exposure, prekeratin was cleaved by 0·05 mol/l NaOH at 25°C to form several soluble derivatives, whereas at comparable time intervals at 4°C both insoluble and soluble derivatives were produced. The various soluble proteins derived from prekeratin showed precipitin bands of identity with the urea-extracted proteins of beef snout epidermis when reacted in agar with antisera raised against the urea-extracted proteins which precipitated at pH 6·3 and 5·5. It is suggested that 6 mol/l urea and 0·05 mol/l NaOH may cleave prekeratin *in situ* to release the proteins extracted by urea and alkali respectively.

Concentrated urea solutions extract from epidermis proteins which precipitate maximally at pH 4·5, 5·5, and 6·3 (Bernstein *et al.*, 1970; Baden & Bonar, 1968; Baden, Bonar & Katz, 1968; Carruthers, 1970; Carruthers *et al.*, 1955a, 1955b, 1957; Rudall, 1952, 1968; Woernley *et al.*, 1957). Matoltsy (1964, 1965) extracted a protein of very limited solubility, called prekeratin, from beef snout epidermis (BNE) with citrate buffer, pH 2·6. Tezuka & Freedburg (1972) have also extracted epidermal structural proteins from newborn rat epidermis with alkaline urea. In this report evidence is presented which indicates that the urea-extractable proteins and prekeratin of BNE are related.

MATERIALS AND METHODS

General and analytical procedures

Procedures for the extraction of the urea-soluble proteins from BNE were given previously (Carruthers, 1972). Prekeratin was extracted from the same tissue by the method of Matoltsy (1964, 1965). Antisera were prepared in rabbits against the urea-extracted BNE proteins which precipitated at pH 6·3 and 5·5 (herein called the urea 6·3 and 5·5 proteins) and were used with the various urea proteins and prekeratin derivatives for immunodiffusion in agar as described before (Carruthers, 1972). Titres of these antisera were determined essentially by the radial immunodiffusion procedure of Masseyeff & Zisswiller (1969).

Separation of the urea proteins and the soluble derivatives of prekeratin was accomplished by gel filtration using Sepharose 4B in the manner indicated previously (Carruthers & Bhattacharya, 1972). The proteins were dialysed against 0·05 mol/l borate buffer containing 0·1 mol/l NaCl (BBS) and the protein content of the dialysed materials was determined (Lowry *et al.*, 1951) prior to chromatography. Protein fractions obtained from the Sepharose 4B columns were concentrated by lyophilization (following dialysis against BBS diluted so that the final buffer concentration in the sample was equivalent to that in the original undiluted BBS), and by ultrafiltration.

Amino acid analyses were carried out on samples of proteins which were hydrolyzed in 6 mol/l HCl under nitrogen for 20 h at 108°C in sealed ampoules. Quantitative amino acid analyses were performed in a Technicon automatic animo acid analyser, using 6·5 h acceleration gradient with norleucine as an internal standard (Roholt & Pressman, 1967).

Fluorescence microscopy was carried out by the indirect procedure (Carruthers & Baumler, 1966). 2 ml of antisera raised against the urea-extractable 5·5 and 6·3 proteins was adsorbed first with 0·2 ml of bovine serum for 30 min at 25°C. The material was centrifuged at 15,000 **g** for 20 min at 0°C. The supernatant fractions were adsorbed successively with sediments (equivalent to 10 ml of a washed 20% tissue homogenate) of bovine liver and kidney in the same manner. They were finally diluted 1:40 with phosphate-buffered sucrose (0·25 mol/l) at pH 7·0 for use in the first step of the indirect procedure, or for further adsorptions with the various urea-extractable proteins, prekeratin, and its soluble derivatives. The protein adsorptions were carried out in the same fashion as the sediments.

The label was prepared by reacting goat anti-rabbit antisera or anti-rabbit gamma-globulins with fluorescein isothiocyanate, and was purified by gel filtration on Sephadex G-50. The active fraction (the first eluted yellow band) was diluted (depending upon staining effectiveness) to 10 or 15 ml with phosphate-buffered 0·1 mol/l NaCl, pH 7·3. The conjugate or label was then adsorbed, like the antisera, with 0·2 ml bovine serum, once with bovine liver and once with bovine kidney sediments. An American Optical Co. Fluorescence Microscope was used, employing a 4 mm Corning 5840 exciter filter and a Schott GG-1 orange–yellow barrier filter.

Preparation of soluble derivatives of prekeratin

Alkali-soluble proteins. 1 mol/l NaOH was added to 80 or 160 mg of prekeratin in citrate buffer until a pH of 10 was reached. Then 15 ml 0·05 mol/l NaOH was added per 80 mg prekeratin, and the mixture, which was solubilized rather quickly, was stirred at 25°C or 4°C with a magnetic stirrer. Aliquots of the alkali-treated proteins were removed at various time intervals, and the pH of the solution was lowered gradually until flocculation of proteins occurred. After settling they were centrifuged at 1800 rev/min for 15 min. The pH of the supernatant fraction was lowered carefully until flocculation again occurred. These proteins were spun down as before. The supernatant fraction was again treated by lowering the pH and, if no further precipitation of proteins occurred, the pH was lowered to about 4, at which pH soluble proteins remained in solution. These alkali proteins are called the alkali 5·8, 5·3, etc. proteins, i.e. the pH at which maximum precipitation occurred. The various alkali proteins were vigorously shaken and stirred (in thick-walled glass centrifuge tubes) with BBS. Solubility was determined by measuring the protein content of the dissolved alkali proteins by the procedure of Lowry *et al.* (1951).

Urea-soluble proteins. The pH of prekeratin in citrate buffer was raised with 1 mol/l NaOH until maximum flocculation of proteins occurred (at about pH 4·5) and, after standing a few minutes, the flocculated protein was precipitated by centrifugation at 1800 rev/min for 15 min. The prekeratin was dissolved in 6 mol/l urea buffered at pH 8·0 with Tris (15 ml urea solution per 80 mg prekeratin). The urea-prekeratin solution was stirred (magnetic stirrer) at 25°C or 37°C. Aliquots of the mixture were removed at various time intervals and dialysed against either barbital buffer (pH 8·6) or BBS to remove the urea. The dialysed material was centrifuged at 1800 rev/min for 10–15 min to remove any insoluble derivatives or original prekeratin. The protein content of the soluble supernatant fraction was determined by the procedure of Lowry *et al.* (1951).

S-carboxymethyl derivatives. The S-carboxymethyl derivatives of prekeratin (CM-derivatives) were prepared from 80 or 320 mg of prekeratin (Carruthers & Bhattacharya, 1972). The prekeratin was precipitated as above and solubilized in 6 mol/l urea buffered at pH with Tris (15 ml urea solution per 80 mg prekeratin). During this process, the pH was maintained between 8·2 and 8·4 before and after the addition of iodoacetate. The derivatives were dialysed against BBS, and the dialysed solution centrifuged at 1800 rev/min for 15 min to remove residual insoluble proteins. The protein content of the supernatant fraction was determined by the procedure of Lowry *et al.* (1951).

Trypsin-solubilized proteins. Two samples of prekeratin (180 and 320 mg) were digested with trypsin. Prekeratin was precipitated as above with 1 mol/l NaOH and the precipitate was thoroughly dispersed in 15 ml borate-buffer, pH 8·5, by homogenization and stirring with a magnetic stirrer at 25°C. The pH was adjusted to 8·2–8·4. Whilst stirring, 0·04 ml of a 4% solution of trypsin was added to the 16 ml solution of protein. After 30 and 60 min of trypsin digestion, 8 ml of the prekeratin solution was removed at each time interval, and then 0·08 ml 3% trypsin inhibitor was added to each 8 ml fraction. Due to the insolubility of the prekeratin at pH 8, trypsinization was difficult to control precisely, but two experiments gave comparable results. The digested prekeratin solution was dialysed against BBS prior to column chromatography and other experiments.

RESULTS

Soluble derivatives of prekeratin

Alkali-derived proteins. The pH at which the NaOH-derived proteins precipitated is given in Table 1. After 46 h of action of 0·05 mol/l NaOH on prekeratin at 25°C, proteins precipitated at pH 5·3, 5·0, and 4·5, and all of these proteins were soluble in BBS. The pH 5·3 protein represented 43% of the prekeratin treated with NaOH, whereas the 5·5 and 4·5 proteins represented 18 and 10% respectively. 30% of the prekeratin remained in solution after lowering the pH to 4·0. Other experiments showed that 0·05 mol/l NaOH at 25°C for periods ranging from 1, 2 or 20 h resulted in the release of proteins precipitated at pH above 5·5 (8·0, 5·7–5·8). These proteins were largely insoluble.

Prekeratin appeared to be degraded more slowly over a period of 120 h by 0·05 mol/l NaOH at 0–4°C (Table 1). 50% of the prekeratin-derivatives were precipitated at pH 6·4 and 5·9; these derivatives would not redissolve. The soluble alkali-derived proteins, which precipitated at pH 5·3 and 4·5, represented 33% of the prekeratin treated with alkali. Also, that part of alkali-derived proteins which were soluble to pH 4·0 was only 18%, as compared with 30% at 25°C.

Urea-soluble derivatives of prekeratin. When prekeratin (80 mg) was dissolved in 6 mol/l urea buffered at pH 8 with Tris and stirred at 25°C for 96, 144, and 259 h, the percentage of prekeratin solubilized was respectively 28, 32, and 41%. Using this solvent at 37°C, 50% of prekeratin was solubilized in 42 h, and complete solution followed after 138 h. 70 mg of the completely solubilized

TABLE 1. pH at which proteins of prekeratin precipitated following solubilization with 0·05 mol/l NaOH

Time of action of NaOH (h)	pH at which proteins were precipitated	Solubility of proteins in BBS	Percent of each protein at pH of precipitation
46*	5·3	Soluble	43
	5·0	Soluble	18
	4·5	Soluble	10
120†	6·4	Insoluble	28
	5·9	Slightly soluble	21
	5·3	Soluble	15
	4·5	Soluble	18

* Digestion of prekeratin carried out at 25°C—30% of these proteins were completely soluble at pH 4·0.

† Digestion of prekeratin carried out at 4°C—18% of these proteins were completely soluble at pH 4·0.

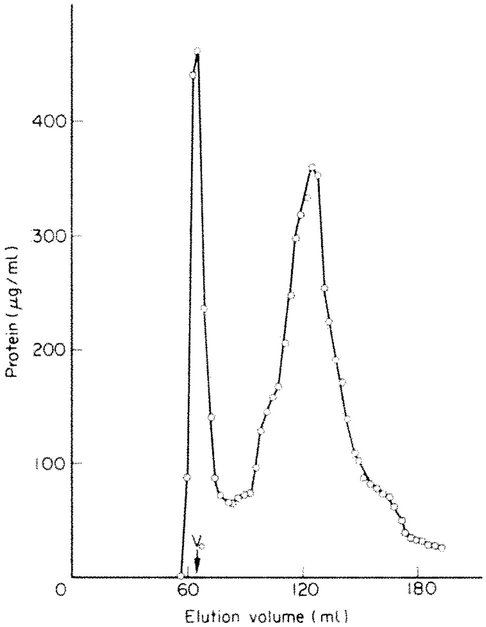

FIGURE 1. Molecular-sieve chromatography (Sepharose 4B) of the CM-prekeratins dialysed against 0·05 mol/l borate buffer pH 8·5 containing 0·1 mol/l NaCl. The protein concentration was monitored during fractionation. The flow rate was 18 ml/h, and V_0, the void volume, was 66 ml.

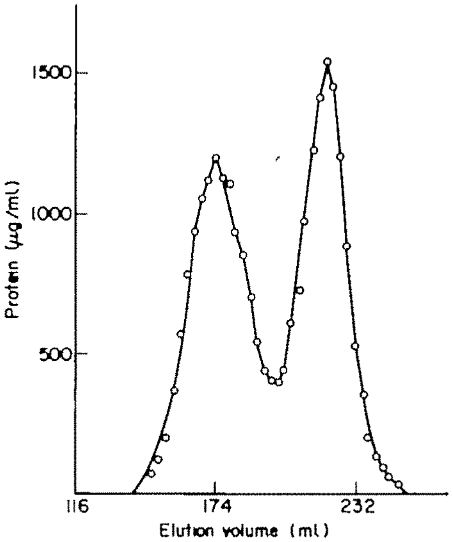

FIGURE 2. Molecular-sieve chromatography as in Fig. 1 of prekeratin treated for 60 min with trypsin. Trypsinized fragments were dialysed against 0·05 mol/l borate buffer, pH 8·5 with 0·1 mol/l NaCl. The flow rate was 16 ml/h, and V_o was 72 ml.

material was dialysed against BBS. The dialysed proteins were separated by gel filtration on Sepharose 4B (with a protein recovery of 98%) resulting in the excluded peak (54% of the protein applied) and a broad retarded peak (46% of the protein applied). These two fractions obtained from the column eluates were concentrated and used for immunodiffusion.

S-carboxymethyl derivatives of prekeratin. CM-prekeratins were dialysed against BBS and separated by gel filtration on Sepharose 4B with a protein recovery of 85%. The elution pattern of these derivatives is shown in Fig. 1. The excluded protein (V_o) represented 42% of the CM-prekeratins applied to the column, whereas the retarded portion was 58% of the total proteins applied to the column. The molecular weight of the proteins of the principal retarded peak was $1{\cdot}4 \times 10^5$, as determined from a calibration curve with pure proteins on Sepharose 4B (Carruthers & Bhattacharya, 1972).

Trypsinized fragments of prekeratin. Prekeratin was trypsinized for 30 and 60 min, and the resulting solutions were dialysed against BBS. The dialysed proteins were then separated by gel filtration on Sepharose 4B with a protein recovery of 98%. The elution pattern of such a sample, which was trypsinized for 60 min, is shown in Fig. 2. A small amount of protein (3·3%) was excluded and was discarded. The first and second well-defined peaks represented 45 and 51·2% of the trypsin derivatives applied. After 30 min digestion the excluded portion of the proteins represented 5·3% of that applied to the column, whereas peaks 1 and 2 with the same elution volumes as those obtained for 60 min accounted for 53·2 and 41·3% of the proteins respectively. The excluded portion of the proteins was discarded. In another experiment, prekeratin was also solubilized by trypsin for 30 and 60 min to give results quite similar to those of the previous experiment. The approximate molecular weights of the first and second peak proteins of both trypsinized samples (30 and 60 min) were $6{\cdot}3 \times 10^4$ and $9{\cdot}7 \times 10^3$ respectively, as determined from a calibration curve (Carruthers & Bhattacharya, 1972) with Sepharose 4B.

TABLE 2. Amino acid composition of epidermal proteins[*]

Amino acid	Urea peak 1 and 2 (av.)[†]	Prekeratin			
		This laboratory	Matoltsy[‡]	Baden[§]	Mean ± standard deviation (urea peak 1 and 2 and prekeratin)
Aspartic	8·7	9·2	8·5	9·1	8·9 ± 0·3
Threonine	3·9	3·8	3·7	4·0	3·8 ± 0·2
Serine	9·9	9·2	10·8	11·1	10·3 ± 0·9
Glutamic	14·0	14·6	16·1	14·1	14·7 ± 1·0
Proline	1·8	1·8	1·5	1·4	1·6 ± 0·2
Glycine	16·1	15·7	14·0	16·4	15·6 ± 1·1
Alanine	7·4	6·8	5·9	6·7	6·7 ± 0·5
Valine	5·4	5·0	4·8	4·0	4·8 ± 1·0
Half-cystine	0·6	0·6	0·8	0·6	0·7 ± 0·1
Methionine	1·3	1·6	0·2	1·3	1·1 ± 0·6
Isoleucine	4·1	4·2	3·9	3·5	3·9 ± 0·3
Leucine	9·0	9·1	8·5	9·2	9·0 ± 0·3
Tyrosine	2·3	2·8	2·2	2·8	2·5 ± 0·3
Phenylalanine	3·6	3·6	3·7	3·6	3·6 ± 0·05
Lysine	5·0	5·0	6·4	5·1	5·4 ± 0·7
Histidine	0·8	0·7	1·2	1·0	0·9 ± 0·2
Arginine	5·9	6·0	7·2	6·1	6·3 ± 0·6

[*] Results expressed as residues per 100 residues.

[†] From Carruthers & Bhattacharya (1972).

[‡] From Matoltsy (1965).

[§] From Baden, Gifford & Goldsmith (1971).

Amino acid analysis. The amino acid composition of prekeratin determined in three different laboratories is given in Table 2; these are in good agreement. The amino acid composition of the BNE urea peak 1 and 2 proteins following chromatography of the urea-extracted 6·3 protein (Carruthers & Bhattacharya, 1972) was in close agreement with that of prekeratin.

Immunodiffusion. Previous experiments showed that the urea-extracted BNE 6·3, 5·5, and 4·5 proteins developed precipitin bands of identity with antisera raised against the 6·3 and 5·5 urea proteins (Carruthers & Bhattacharya, 1972). The titres of the antisera produced against the 5·5 and 6·3 proteins were respectively 1:256 and 1:512. Also, the following proteins reacted with the same antisera to develop precipitin bands of identity with the BNE 6·3, 5·5 and 4·5 proteins: (1) CM-prekeratins peaks 1 and 2, Fig. 1; (2) trypsin digests peaks 1 and 2, Fig. 2; (3) retarded and excluded peaks of the urea-37°C extract; and (4) the alkali soluble proteins which precipitated maximally at pH 5·3, 5·0, and 4·5. The diffusion patterns of the various prekeratin derivatives were similar to those presented previously for the urea-extracted BNE proteins (Carruthers & Bhattacharya, 1972).

Further evidence that the urea-soluble and CM-prekeratins are related to the urea-extracted BNE proteins was obtained by inhibition of precipitation band formation in immunodiffusion by adsorption of both antisera with the urea-extractable and prekeratin derivatives listed above.

In other experiments were determined the amounts of the various urea-extractable proteins, prekeratin, and some of its soluble derivatives required to abolish fluorescence on sections of beef snout epidermis by the fluorescent antibody technique with the antisera raised against the urea-extractable

TABLE 3. Levels of urea 4·5, 5·5, and 6·3 BNE proteins, prekeratin and its soluble derivatives required to quench fluorescence from antisera raised against the urea 5·5 and 6·3 BNE proteins

Proteins used to inhibit fluorescence	Antisera* employed and mg proteins required to inhibit fluorescence (mg protein per 2 ml antisera)	
	With urea 5·5 protein antisera	With urea 6·3 protein antisera
4·5 BNE proteins	1·4	0·9
5·5 BNE proteins	0·3	0·4
6·3 BNE proteins	0·9	0·8
Prekeratin†	0·4	0·4
CM-prekeratins	0·2	0·2
Trypsin derivatives of prekeratin		
30 min peak 1 proteins	1·2	1·0
30 min peak 2 proteins		1·0
60 min peak 1 proteins	0·5	0·4
60 min peak 2 proteins	0·4	0·6

* Antisera were first adsorbed with bovine serum, then with liver and kidney sediments and diluted 1:40 with phosphate-buffered sucrose (0·25 mol/l sucrose), pH 7·0, for fluorescence microscopy by the indirect procedure. Then 2 ml of the diluted serum- and tissue-adsorbed antisera were adsorbed with increasing levels of the various proteins until fluorescence was abolished.

† Prekeratin was dissolved in 6 mol/l urea.

5·5 and 6·3 BNE proteins. For this purpose these antisera were adsorbed and diluted as described above. In order to determine the amount of BNE urea-extracted 4·5, 5·5, and 6·3 proteins, prekeratin, and the prekeratin soluble derivatives necessary to remove the antibodies capable of staining BNE, 2 ml of the diluted and adsorbed antisera were treated with increasing levels of these proteins until the fluorescence on BNE was abolished. It is apparent from Table 3 that the amounts of each of the urea 4·5, 5·5, and 6·3 urea proteins, prekeratin and its soluble derivatives required to abolish fluorescence from the 5·5 and 6·3 urea protein antisera were appreciably different, but approximately the same for each antiserum.

DISCUSSION

The results of the experiments reported in this paper on the solubilized derivatives of prekeratin by various chemical procedures revealed that these derived proteins contained immunochemically identical components to the urea BNE-extractable proteins. Thus antisera raised against the BNE 6·5 and 5·5 urea proteins developed precipitin bands of identity with the various solubilized prekeratin proteins in immunodiffusion in agar, and inhibition of precipitin band formation was accomplished by absorbing the BNE 6·5 and 5·5 protein antisera with the solubilized prekeratin proteins. Also, employing the fluorescence antibody technique on tissue sections of BNE, fluorescence was abolished by adsorption of the 6·5 and 5·5 urea protein antisera with the various solubilized prekeratin proteins and prekeratin itself.

In this connection Bauer (1972) showed that antibodies prepared against the alkali 5·5 proteins of human epidermis gave an immunodiffusion precipitin band of identity with the alkali 5·0 and 4·5 proteins, thus indicating the presence of immunologically identical components on these proteins. Also Tezuka & Freedberg (1972) demonstrated that alkaline urea extracts of newborn rat epidermis gave three proteins which exist in several states of polymerization. Each of the polymeric forms contained an immunologically identical component.

The results reported in this paper suggest that the high molecular weight prekeratin of Matoltsy (1964, 1965) is solubilized by both urea and alkali *in situ*, but in the process prekeratin is degraded to sub-units which vary widely in molecular weight. Although dilute alkali, urea, S-carboxymethylation, and trypsin yield sub-units from prekeratin which have common antigenic determinants, more data is required to demonstrate whether these proteins have different degrees of aggregation of a basic sub-unit, or whether these sub-units vary in conformation. Since prekeratin was solubilized by several different reagents, and yet the solubilized proteins showed immunochemical identical components, there is probably a different number of sub-units present in solubilized prekeratin. This assumption is supported by the fact that the CM-prekeratins consisted of a high molecular weight fraction (Fig. 1, excluded protein), and another protein with a molecular weight of $1{\cdot}4 \times 10^5$. On the other hand, prekeratin solubilized by trypsin yielded soluble proteins of prekeratin with molecular weights of $6{\cdot}3 \times 10^4$ and $9{\cdot}7 \times 10^3$ (Fig. 2), yet these four proteins also contain immunologically identical components. Thus, it would appear that prekeratin contains protein segments, cleaved at various loci by the reagents employed to give the proteins, which have an immunochemically identical component. The possibility exists that each of these derived prekeratin proteins represent various stages in the aggregation process which leads to the formation of prekeratin.

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Reduction of sebum excretion in man by the antiandrogen, cyproterone acetate

J.L.BURTON*, URSULA LASCHET† AND SAM SHUSTER

Wellcome Laboratories for Research into Skin Disease, University Department of Dermatology,
Newcastle-upon-Tyne and †Pfalzische Nervenklinik, Psychendokrinologische Abteilung,
6749 Landeck/Pfalz, Germany

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SUMMARY

The effect of systemic cyproterone acetate on sebaceous activity was studied in twenty male patients being tested for sexual offences. In every patient the sebum excretion rate (SER) was below the mean SER of untreated normal male subjects of the same age, and the mean SER for the treated male patients was significantly lower than in a control group of normal females. The degree of reduction in SER produced by cyproterone acetate confirms that androgen is a major sebotrophic factor in the human male.

The antiandrogen cyproterone acetate decreases sebum secretion in the rat (Archibald & Shuster, 1967, 1969), and reduces sebaceous gland volume in the mouse (Neumann & Elgar, 1966). Cunliffe, Shuster & Cassels-Smith (1969) reported that cyproterone acetate applied topically in DMSO was without effect on sebum secretion, but to our knowledge the effect of systemic cyproterone acetate on sebum production in man has not been measured. We have now studied the effect of systemic cyproterone acetate in male patients who were being treated for sexual offences.

METHOD

We studied twenty male patients, aged 22–53, who were attending a psychiatric hospital in Germany for the treatment of sexual offences, such as exhibitionism, pederasty and rape. Every patient was receiving systemic cyproterone acetate in a dose ranging from 25 to 200 mg daily (mean 100) and the duration of continuous therapy ranged from 2 to 75 months (mean 37). The following investigations were done before and during treatment: urinary 17-keto- and 17-ketogenic steroids, plasma cortisol, total oestrogens, interstitial-cell stimulating hormone, total gonadotrophic activity, and FSH. Every patient also had a glucose tolerance test, and most patients had a sperm count and sex chromatin or chromosome analysis. The results of these investigations and treatment have been reported elsewhere (Laschet & Laschet, 1969a, 1969b, 1971).

The sebum excretion rate (SER) in forehead skin was measured in every patient by the method of Strauss & Pochi (1961) as modified by Cunliffe & Shuster (1969). The sebum specimens were collected in Germany, stored in glass bottles at −20°C for not more than 48 hr and then put in an ice-box and flown to England for measurement. Because no new patients were started on treatment with cyproterone acetate at the time of the study, it was not possible to measure the SER before as well as during treatment. For comparison, the SER was also measured in forty-three male and sixty-four female patients, aged 20–60, who were attending the Skin Department in Newcastle-upon-Tyne for treatment of benign localized dermatoses, such as tinea pedis and warts, but who were otherwise normal.

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* Present address: Department of Dermatology, Royal Infirmary, Bristol.

RESULTS

The SER of the individual patients taking cyproterone acetate was in every case below the mean SER of the male control subjects (Fig. 1). The mean SER for the twenty patients taking cyproterone acetate was 0·37 ± SE 0·05 $\mu g/cm^2/min$. From our measurements in normal subjects the mean SER for twenty normal male subjects of the same ages as the patients receiving cyproterone acetate would be 1·33 ± SE 0·03 $\mu g/cm^2/min$ and the corresponding value for twenty normal females would be 0·62 ± SE 0·02 $\mu g/cm^2/min$. The mean SER in the cyproterone acetate treated patients was very significantly less than the values in both the male and the female control subjects ($P < 0{\cdot}001$). When the SERs of the cyproterone patients were expressed as a percentage of the SERs of the male control subjects of the same age, the mean SER was 28 ± SE 4% of the normal value. The size of the decrease in SER in the individual patients receiving cyproterone acetate was not related to the duration of therapy ($r = 0{\cdot}04$).

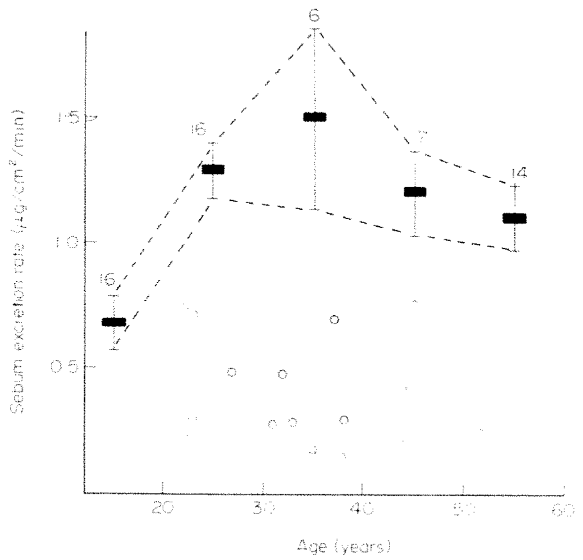

FIGURE 1. SERs of patients and controls. ■, controls (mean ± SE); ○, cyproterone acetate patients.

DISCUSSION

We have found that the SER is greatly reduced in patients receiving systemic cyproterone acetate therapy for psychosexual deviation and that this reduction persists while the treatment is continued. Because we were unable to measure the SER before and after treatment in individual patients we cannot exclude the possibility that all the patients studied had a reduced SER before treatment. We feel this is unlikely, however, as they had a normal output of male androgen metabolites before treatment and there was no other endocrine disturbance, such as hypothyroidism or hypopituitarism, likely to reduce sebum production. A reduction in sebum excretion due to cyproterone acetate alone has not previously been reported in man, though several authors have noted that when combined with oestrogen, cyproterone acetate will reduce seborrhoea in hirsute women (Hammerstein & Cupceancu, 1969; Neale, Krebs & Bettendorf, 1971; Leo-Rossberg *et al.*, 1971). The clinical response of hirsutism to cyproterone acetate is superior to cyproterone, the free alcohol (Hammerstein & Cupceancu, 1969). Our present results suggest that cyproterone acetate is also more effective than cyproterone in reducing sebum production, for the decrease we observed after cyproterone acetate was greater than the decrease in sebum production which was produced by cyproterone (Winkler, 1968; Tamm *et al.*, 1970).

Many hormones are known to stimulate sebaceous gland function in rat and man; the degree to which antiandrogen reduced the SER in the present study confirms that androgen is a major sebotrophic factor in the human male.

The antiandrogen cyproterone acetate has now been used at the Pfälzische Nervenklinik for the treatment of sexual disorders for 6 years. There have been no serious side-effects, and the suppression of sexuality which the drug produces appears to be reversible on stopping the treatment. The suppression of libido and the possible effects on developing gonads and sex hormone dependent tissues probably preclude the systemic use of this antiandrogen for skin disorders, but further study of the topical application of this drug in conditions such as female hirsutism, acne and male-pattern baldness would be worthwhile.

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A rapid increase in skin extensibility due to prednisolone

J.L.BURTON AND SAM SHUSTER

Wellcome Laboratories for Research into Skin Disease, University Department of Dermatology,
Newcastle-upon-Tyne

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SUMMARY

We have tested the short-term effect of prednisolone on the ability of the skin to withstand a shearing stress, using a specially designed stretching machine. A single intravenous infusion of 2 g prednisolone given to seven patients with alopecia totalis produced an increase in skin extensibility within 2 h which persisted for at least a week. This acute effect could be due to a change in dermal ground substance or to breaking of collagen crosslinks. We suggest that patients receiving very large doses of glucocorticoids should avoid undue exertion during, and for some time after, treatment, since a similar effect might occur in the connective tissue of other organs, such as the aorta.

The systemic infusion of a single huge dose of glucocorticoids has been used recently to suppress the rejection of renal transplants (Kountz & Cohn, 1969; Bell *et al.*, 1971). The treatment appeared to be free from side-effects, and it seemed that this therapeutic regimen might be useful for other conditions in which corticosteroids produce a beneficial effect. Alopecia areata is one such disease, and so we studied the therapeutic effect of a single intravenous infusion of 2 g prednisolone in alopecia totalis (Burton & Shuster, to be published). The treatment produced no clinical side-effects, except that one patient developed striae on the thighs within 4 days of the infusion. Skin resistance to stretch is due mainly to collagen, but while prolonged administration of glucocorticoids is known to affect dermal collagen content (Scarborough & Shuster, 1960; Shuster, Raffle & Bottoms, 1967; Black, Shuster & Bottoms, 1973), little is known of a possible acute effect. We have, therefore, now tested the short-term effect of prednisolone on the ability of the skin to withstand a shearing stress.

METHOD

We studied seven patients aged 18–65 years who were well-apart from alopecia totalis. Each patient was treated with a single intravenous infusion of 2 g prednisolone in 200 ml saline over 2 h. The ability of the forearm skin to withstand a shearing stress was measured before treatment and at 2 h, 24 h, 3 days, 7 days, and 12 weeks after the end of the infusion.

The measurements were made with a machine which had been specially designed to assess the response of human skin to stretching *in vivo* (Fogarasy & Shuster, to be published). The machine

Reprint requests to Dr J.L.Burton, Department of Dermatology, Bristol Royal Infirmary, Bristol.

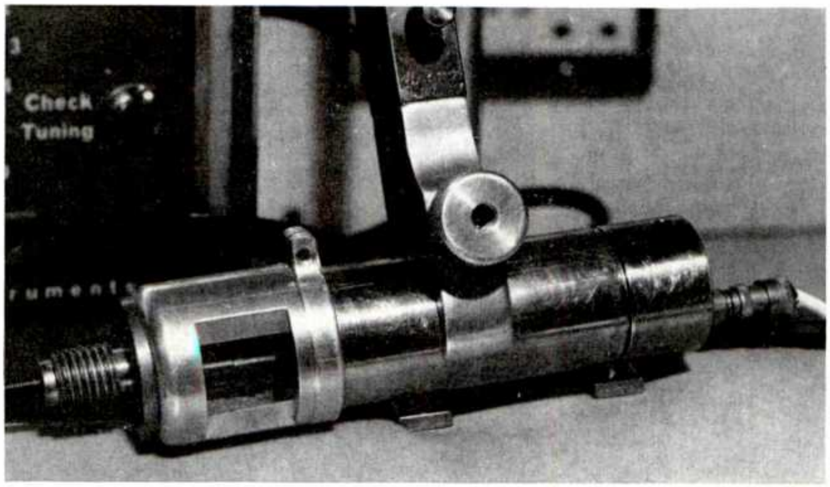

FIGURE 1. Electrically driven stretching head *in situ*.

consisted of an electrically driven stretching head (Fig. 1) and associated electronic equipment for recording the force/extension characteristic. The stretching head carried two square metal feet which were stuck to the skin with Eastman 910 adhesive cement and were then driven apart by a motor system with a flexible drive. The motor control system was compensated so that any selected speed could be held constant despite torque fluctuations. One metal foot, mounted upon a spindle and driven by the motor, had a travel of 30 mm. The other foot was attached to a very stiff metal spring which underwent a small deflection when a load was applied, the deflection being sensed by an inductive proximity transducer connected to an oscillator and amplifier. The output signal, which was proportional to the deflection of the spring, was recorded on a Servoscribe pen recorder against a time base. The force recording system was calibrated by comparing the recorder signal with a known load applied directly to the foot carrying the spring.

All the measurements were made on the flexor aspects of the forearm in a longitudinal direction. The area was shaved and the 1 cm^2 metal feet were then stuck in position with a 2 cm gap between their inner edges. The feet were then fitted into the stretching head and, after zeroing the amplifier and recorder, the motor and recorder were started. Stretching was continued until the feet slipped due to detachment of the stratum corneum or until the patient experienced pain. Each measurement was performed on two test sites which were arranged in pairs, one site being distal and the other proximal, to compensate for possible differences in skin extensibility along the forearm; and the mean of the slopes at the two sites was recorded. The same test site was not used twice, since stretching the skin modifies subsequent responses at this site, and the order of testing was randomized.

The load-extension curve produced by this stretching procedure is shown in Fig. 2. The initial flat slope represents the taking up of the slack in the skin. The steep fall at the end of the curve is due to the stratum corneum yielding under stress, since at this point the feet are seen to slip, and the stratum corneum is found to be stuck to the adhesive cement when the feet are removed. The rectilinear slope in the middle of the curve is more difficult to interpret. The dermal collagen fibres are initially orientated in three dimensions (Finlay, 1969), though there is probably a pattern of alignment in a particular direction in any given area depending on the predominant stress which normally obtains. As the slack is taken up, the fibres begin to align into parallel bundles along the direction of stretch. Although the precise relationship between load and alignment is unknown, there is probably

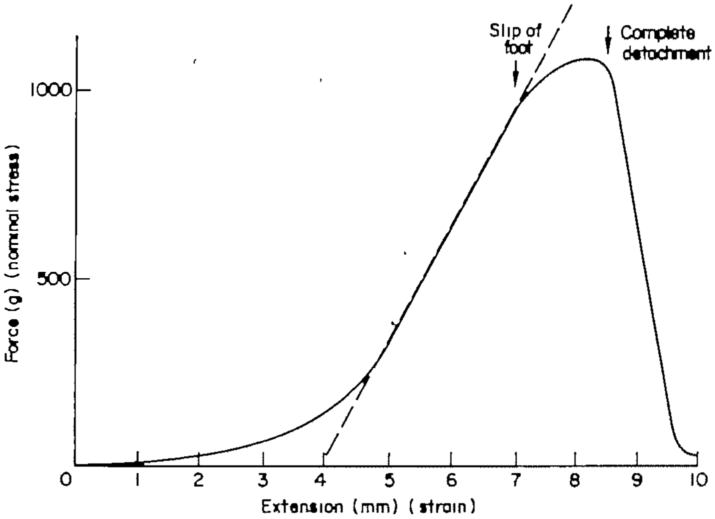

FIGURE 2. Force/extension curve for forearm skin. Slope of rectilinear portion of curve = nominal stress/strain.

a gradual recruitment of fibres as the stress is increased, and the fibres will not all be optimally aligned until the dermal collagen is almost on the point of rupturing. A probable explanation for the load–extension curve, therefore, is that as the fibres become parallel the curve steepens, and becomes rectilinear as the applied stress is resisted by the fibres, which gradually yield.

The slope of the rectilinear portion of the curve was thus measured as the quotient of the force applied divided by the extension produced by separation of the feet, and is thus equivalent to 'nominal stress/strain', which is proportional to Young's Modulus.

RESULTS

The slopes of the rectilinear portions of the force–extension curves (mean ± SE for the group) show that the skin extensibility began to increase within 2 h of the end of the prednisolone infusion and continued to increase for 3–7 days, but had returned to normal by 12 weeks later (Fig. 3). If the slopes for each patient are expressed as a percentage of the slope before prednisolone infusion, the mean slopes for the group are very significantly decreased at 1, 3 and 7 days ($P<0{\cdot}01$), the mean slope at 3 days being 68·5 ± SE 2·7% of the initial mean slope.

DISCUSSION

Our results show that prednisolone infusion in high dosage causes an increase in skin extensibility which begins within 2 h, and the increase persists for at least 7 days. The ability of the skin to resist a shearing stress is thought to depend largely on the extensibility of the collagenous dermis. Houck has claimed that large amounts of insoluble collagen disappear from rat skin within 24 h of the administration of cortisol (Houck, 1962; Houck, Patel & Gladner, 1967). The amounts lost were too great to be accounted for by the inhibition of anabolism, and the effect was thought to be due to the increased collagenase activity induced by stress or steroid administration in the rat (Houck *et al.*, 1968, 1970). This work has not yet been confirmed in other laboratories, however, and Bottoms & Shuster (to be published) found that, while glucocorticoids led to a rapid increase in soluble collagen in

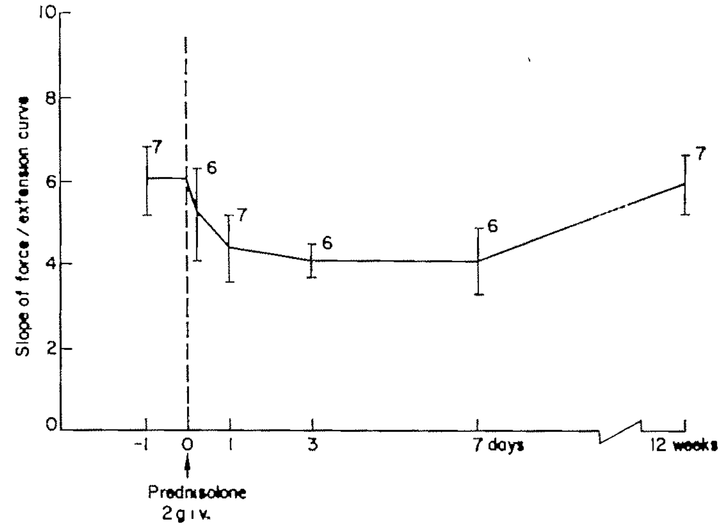

FIGURE 3. Slope of the force/extension curve plotted against time.

the days after corticosteroid administration, there was no change in the insoluble mature collagen, which constitutes the bulk of the collagenous dermis. The increased skin extensibility we observed after prednisolone probably occurred too quickly for the effect to be due to either increased catabolism or decreased synthesis of collagen.

Glucocorticoids affect various constituents of the cutaneous ground substance within hours or days (Thomas, 1956; Smith, 1962; Fediay & Clay, 1964). Since ground substance would be displaced as collagen fibres are realigned by stretching, it seems likely that a decrease in ground substance viscosity could reduce the dermal resistance to stretching. In preliminary experiments in four of our patients, however, the rate of disappearance of an intradermal weal produced by the injection of 0·1 ml of normal saline was not affected by the prednisolone infusion, and this suggests that no dramatic change in ground substance viscosity had occurred. The resistance of the dermis to stretch depends also on the crosslinks within the collagen fibrils (Bottoms, Cater & Shuster, 1966) and the possibility that corticosteroids increase skin extensibility by breaking collagen crosslinks also requires further testing.

The infusion of large doses of glucocorticoids has been used successfully to suppress the rejection of organ transplants (Kountz & Cohn, 1969; Bell *et al.*, 1971) and this therapeutic regimen might become widely used in other conditions in which steroids have a beneficial effect. Our present experiments show that prednisolone in high doses is capable of rapidly increasing skin extensibility, and this, together with our clinical observation that one patient developed striae within 4 days of the infusion, suggests that this treatment requires careful supervision. A similar effect might be produced on internal connective tissue, and no stretch of the imagination is needed to predict the effect of a rapid decrease in aortic tensile strength. The chest radiographs in our patients showed no change in aortic arch width after treatment, but until this point has been investigated further it would be wise for patients receiving very large doses of systemic corticosteroids to avoid undue exertion for at least a week after the infusion.

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Induction of epithelial hyperplasia by *Candida albicans*

R.A.CAWSON

Department of Oral Medicine and Pathology, Guy's Hospital Medical School, London SE1 9RT

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SUMMARY

Epithelial hyperplasia is a characteristic feature of mucosal infection by *Candida albicans*, but doubt may exist as to the extent to which this reaction is a direct result of fungal invasion. An experimental model using the chick embryo chorio-allantoic membrane infected by *C. albicans* shows an essentially similar hyperplastic response of the ectoderm, and several features in common with human infections. It is suggested that the intracellular growth of *C. albicans* in epithelium is probably a major factor provoking this response.

Invasion of an epithelial surface by *Candida* species is common in the mouth and is well known in acute form as thrush. There are also chronic varieties of 'candida leukoplakia' which can develop either as isolated lesions or as a feature of muco-cutaneous candidiasis. The feature common to these lesions is epithelial hyperplasia, and it is important to know whether this is a direct response to invasion by candida or whether, as Jepson & Winther (1965) have suggested, that candida infection becomes superimposed on hyperplastic epithelial plaques. Until recently there has been no satisfactory experimental model to study the pattern of formation of these epithelial plaques. Partridge, Athar & Winner (1971), however, used inoculation of the chorio-allantoic membrane of the chick embryo in another context, i.e. as a test of pathogenicity of candida isolates. In this model hyperplastic epithelial plaques are formed, and the purpose of this paper is to draw attention to the histological changes which accompany tissue invasion in this experimental model and to compare them with clinical candida lesions in the human subject.

MATERIAL AND METHODS

The method of inoculating a chorio-allantoic membrane (CAM) has been described (Partridge, 1959), but in brief, 10-day embryonated chickens' eggs are used, the CAM is 'dropped' and an inoculum of 0·1 ml 10^6 *Candida* cells is introduced through the outer membrane on to the CAM and the opening sealed. The CAM is removed after periods of 2–4 days incubation, in most cases at 37°C. Creamy, white plaques, usually 2–3 mm in diameter, were then visible. The CAM was fixed in formol saline and processed in routine fashion for sectioning. Human material for comparison was taken from lesions of chronic oral candidiasis using standard biopsy procedures and routine processing methods.

RESULTS

Histological changes

The ectoderm of the CAM is normally only 1 or 2 cells thick, and this delicate layer is not closely comparable with human oral mucosa in either structure or function. Following inoculation, the first sign of invasion histologically is hyperplasia and stratification of the ectoderm, which comes closely to resemble stratified squamous epithelium. The plaque progressively thickens and reaches a maximum of about 6–18 cells in thickness; the cells are nucleated throughout the full thickness, including the surface. Most superficially there are some amorphous debris and numbers of candida. On the surface these are predominantly in the yeast form, while hyphae can be seen invading the upper layers of the plaque. Penetration does not usually extend into more than about half the thickness of the plaque until the pre-terminal stages, when invasion of the mesoderm takes place. In addition, there is some oedema of the superficial layers of ectoderm and varying degrees of separation of the cells of this part of the plaque. Some inflammatory cells, apparently mononuclear cells or histiocytes, can be seen both in the superficial epithelium, where occasional micro-abscesses form, and in smaller numbers in the underlying mesoderm. The proliferation of the epithelium is 'upwards', i.e. there is no deep proliferation (acanthosis), as seen in human lesions. In the pre-terminal stage, however, just before the death of the embryo, there is downward proliferation of the ectoderm into the mesoderm, followed by invasion and massive proliferation of the fungus into the deeper tissues with gross abscess formation.

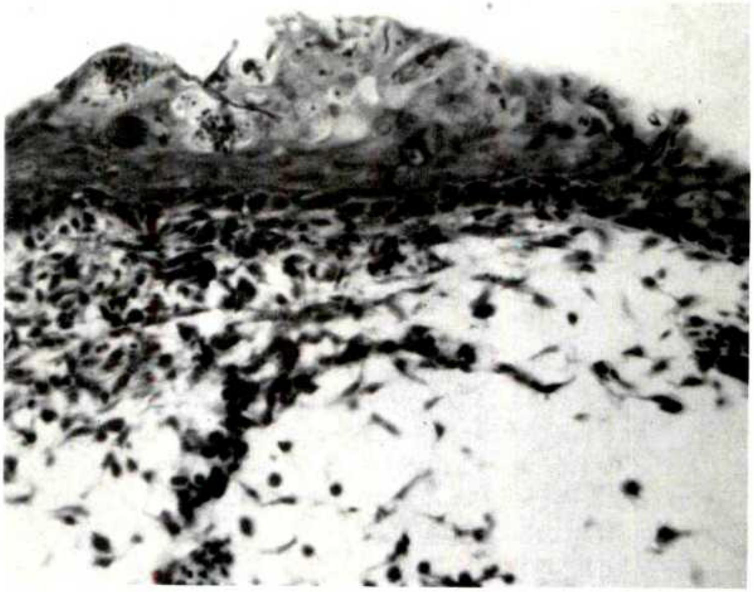

FIGURE 1. Early infection of chorio-allantoic membrane showing epithelial plaque formation (PAS, × 375).

Histological changes of human muco-cutaneous candidiasis

These changes have been described by Jepson & Winther (1965), Cawson (1965, 1966), and Cawson & Lehner (1968). The prominent features common to both acute and chronic invasive candidiasis of the

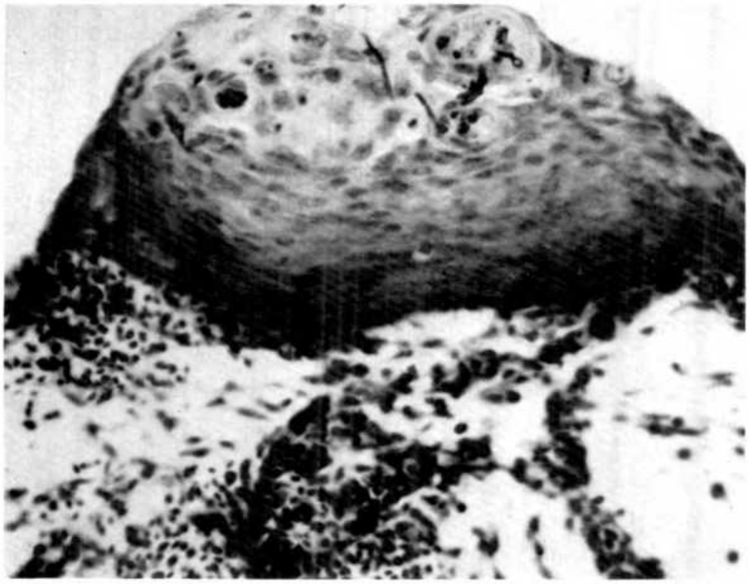

FIGURE 2. Candida infection of CAM showing more vigorous epithelial response, and differentiation of the ectoderm to a stratified squamous type (PAS, × 375).

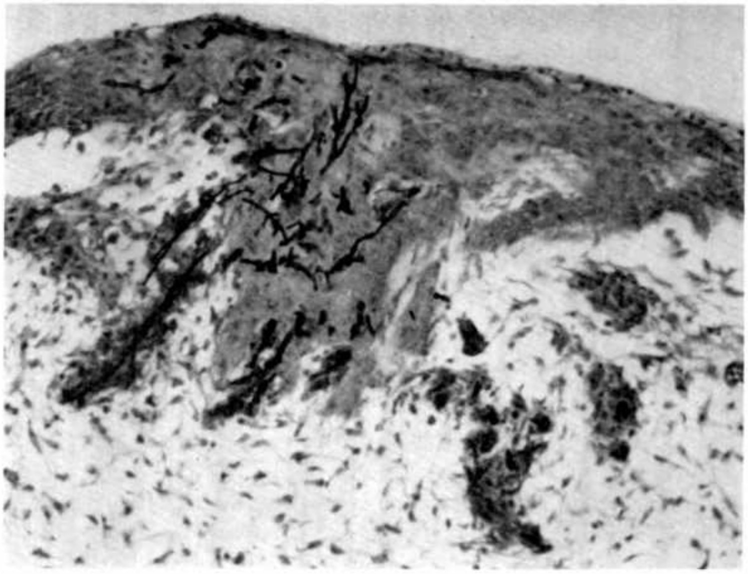

FIGURE 3. Late candida infection of CAM with downgrowth of epithelium accompanying deep invasion by hyphae just before dissemination (PAS, × 240).

oral mucosa are; (a) hyperplasia of the superficial epithelium invaded by hyphae, (b) proliferation of the deeper spinous layer, and (c) an inflammatory infiltrate both in the superficial plaque and in the corium. The hyphae extend downwards through the epithelium, but reach only to the glycogen-rich zone. This zone of invasion is also characterized by epithelial hyperplasia, which is apparent clinically as plaques varying from minute 2 mm flecks in mild thrush to extensive confluent plaques several centimetres across. The superficial inflammatory exudate within the infected plaque is of characteristic appearance. There are large numbers of polymorphonuclear leukocytes and mononuclear cells in close relation to the invading hyphae, so numerous sometimes as to form micro-abscesses within the epithelial plaque. In addition, there is inflammatory oedema, which may be seen as clear beads separating the epithelial cells or may be gross and extensive, allowing the plaque to separate easily from the deeper epithelium.

Beneath the infected plaque, that is to say, deep to the glycogen-rich region of the epithelium, there is epithelial hyperplasia with varying degrees of acanthosis. Inflammatory cells are few or absent in this area. In the underlying corium there is a mixed inflammatory infiltrate with varying numbers of plasma cells, lymphocytes, and other mononuclear cells.

Though the development of hyperplastic epithelial plaques is common to both acute and chronic candidiasis there are important differences. In thrush, candida are exceedingly numerous in the infected plaque and the inflammatory changes are florid, so much so that the oedema extends through its whole thickness giving it the soft friable character which is its clinical characteristic. Oedema and abscess formation are particularly severe just beneath the limit of invasion by hyphae at the junction with the deeper epithelium, so that there is progressive separation of the plaque or the plaque may be easily rubbed off, exposing the deeper epithelium. In chronic candidiasis by contrast, the plaques are firm and cannot be rubbed off and hence resemble leukoplakia due to other causes. There are relatively smaller numbers of organisms and more localized superficial inflammatory changes, but much more massive, deep hyperplasia, often with acanthosis of gross degree.

Common features between experimental and clinical infection

Looking at the plaque itself, there are close similarities between human candidiasis and the experimental infection. In both, there is an obvious hyperplastic epithelial plaque invaded by hyphae of candida and with varying degrees of oedema and infiltration by inflammatory cells near the surface. In plaques formed on the CAM, however, inflammatory changes within the epithelium, and for that matter in the corium, are less prominent than in the human subject, and there is also no downward proliferation (acanthosis) of the ectoderm until the sub-terminal stages. The plaque on CAM is also, of course, of limited extent. The illustrations show these common features and the differences.

Progress of superficial candidiasis

In the human subject an acute candida infection usually clears up either apparently spontaneously or as a result of treatment. In a small minority of patients thrush in childhood persists and the condition of chronic muco-cutaneous candidiasis becomes established. This may or may not be associated with endocrine disturbance, notably hypoparathyroidism, and in many of these patients a genetic background is evident (Higgs & Wells, 1972). In other patients, however, chronic hyperplastic candidiasis (candida leukoplakia) becomes established, apparently late in life, and as in children with muco-cutaneous candidiasis the infection is long-lasting and intractable. In the chick embryo, by contrast, the infection persists for a maximum of about 6 days, or occasionally a few days longer, but thereafter the embryo dies, especially when infected with virulent candida strains. As a result only the earlier stages of candida invasion and plaque formation can be followed. Death is usually preceded by downgrowth of the ectoderm into the mesenchyme with massive proliferation of and

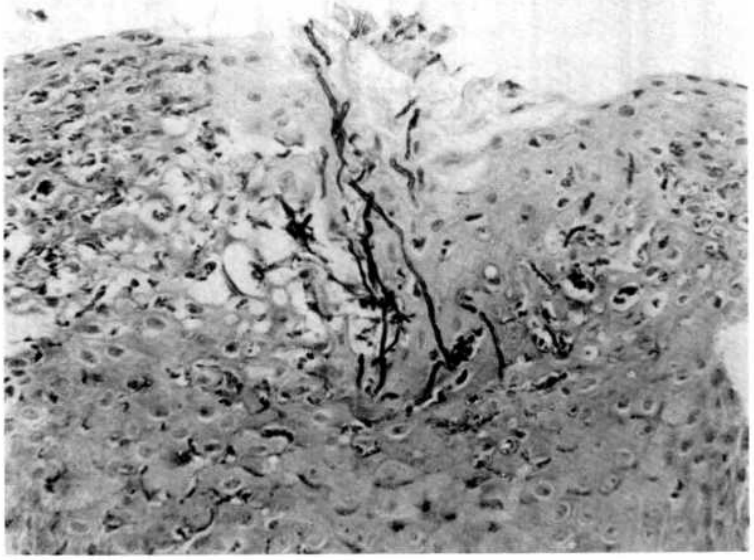

FIGURE 4. Human mucocutaneous candidiasis. This infection is of several years' standing and shows more massive epithelial hyperplasia than in the experimental infection (PAS, × 250).

invasion by the fungus and abscess formation. In general, however, the older the embryo at the time of inoculation, the less likely it is to succumb.

DISCUSSION

Bearing in mind that the CAM is primarily respiratory in function and is not strictly comparable with oral epithelium in man, the histological changes of invasive candidiasis are surprisingly similar. Differences in the histological appearances can be largely attributed to the different natural function of this chick ectoderm. The different natural history of the disease must also be determined to a large extent by the much greater defensive powers of the human subject than those of the immature chick embryo. In man, therefore, *C. albicans* is frequently present in the mouth as a commensal. In those patients in whom chronic hyperplastic mucosal or muco-cutaneous candidiasis develops, a balance between infection and reaction appears to become established. In these cases there is severe, widespread, and intractable plaque formation caused by invasion of mucous membranes and skin persisting for many years, but the infection remains superficial. If on the other hand candidas enter the blood stream, as may happen for instance during cardiac surgery, candida endocarditis or septicaemia can develop and is life-threatening.

In both man and the chick embryo, therefore, the situation exists that while candida infection is within epithelium there is an epithelial and inflammatory response which remains localized and the subject remains alive and well. When *C. albicans* invades deeper tissues, however, or reaches the blood stream, the infection is invariably fatal in the chick embryo and often also in man. While immunological mechanisms must play some part in resistance to candida infection it appears that they are less effective once the organism reaches the blood stream. Further, although candidas are able actively to invade epithelium it is noticeable that in human infections the hyphae remain restricted in their depth of penetration.

The significance of this experimental infection is, therefore, that it shows clearly that the invasion of an ectoderm by *C. albicans* induces epithelial hyperplasia where the infection remains localized. Norris, Shorey & Bongiovanni (1948) showed that only live organisms produced this effect, and that killed organisms do not elicit any response by the CAM. Further, Partridge *et al.* (1971) have shown that epithelial plaque formation is directly related to the pathogenicity of candida isolates.

It may be said that this response of epithelium to candida infection may be obvious from the histology in man, but this feature does not seem to have received the attention it deserves. As mentioned earlier, Winther & Jepson (1965) thought that chronic plaques showing candida hyphae were due to candida infection superimposed on a pre-existing lesion, though in fact no evidence of pre-existence of these lesions was brought forward.

As to determinants of this response of epithelia to invasion by *C. albicans*, the findings of Montes & Wilborn (1968) are relevant. These workers reported, and it has since been confirmed (Cawson & Rajasingham, 1972), that electron microscopy shows that the growth of *C. albicans* in the lesions of candidiasis is actually that of an intra-cellular parasite of the epithelial cell.

Since the experimental evidence indicates that candida infection promotes epithelial hyperplasia, and since chronic candidiasis can give rise in man to oral leukoplakia (Cawson, 1966; Cawson & Lehner, 1968), it is important to consider whether this process can go further to malignant change. Renstrup (1970) in fact found that 61% of so-called speckled leukoplakias showed candida invasion, and 71% of these were characterized by epithelial atypia in addition to proliferation. Later, Roed-Petersen, Renstrup & Pindborg (1970) found that 40% of all candida leukoplakias showed epithelial atypia. Robinson & Tasker (1947) suggested that chronic candidiasis might be pre cancerous, and there have been a number of reports of carcinoma developing in relation to lesions of chronic candidiasis. The earliest of these was that of Engman & Weiss (1920), while more recently Kugelman, Cripps & Harrell (1963), Jansen, Dilaha & Honeycutt (1963), Williamson (1969), and Eyre & Nally (1971) have reported cases. It is probable that the small number of reported cases has been due to the common failure, which Robinson & Tasker (1947) emphasized, to recognize chronic candidiasis in the past. Nevertheless, it is essential to appreciate that these reports only describe an association between chronic candidiasis and carcinoma. There is in fact no clear evidence that chronic candidiasis in itself is pre-cancerous, but it is possible that invasion of the epithelial cell by this organism and the resulting cellular proliferation could, in association with other as yet unknown factors, contribute to neoplastic change.

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Pharmacology and Treatment

A trial of hydrocortisone butyrate in the treatment of rosacea and perioral dermatitis

IAN SNEDDON

Rupert Hallam Department of Dermatology, Sheffield

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SUMMARY

In a blind trial a non-fluorinated steroid, 0·1% hydrocortisone butyrate, was compared with 1% hydrocortisone alcohol on twenty-eight patients suffering from steroid induced atrophy superimposed on rosacea and perioral dermatitis.

The hydrocortisone butyrate did not behave like a fluorinated steroid, in that it did not prevent the beneficial effect of tetracycline nor the disappearance of telangiectasis and atrophy. In twenty-eight patients, hydrocortisone butyrate was superior in fifteen, in six hydrocortisone alcohol, and in seven the results were equal.

It is concluded that although hydrocortisone butyrate is as effective as triamcinolone acetonide or fluocinolone in the treatment of psoriasis and eczema, it does not prevent the recovery of steroid atrophy on the face, and thus may not even cause it.

It is now well recognized that the repeated application of fluorinated steroids will produce damage to dermal collagen (Epstein, Epstein & Epstein, 1963; Grice, 1966; Ive & Marks, 1968), which is indicated by telangiectasia. This change is particularly noticeable in patients with rosacea who have used topical fluorinated steroids for over 6 months (Sneddon, 1969). Cessation of the use of fluorinated steroids on the face induces a rebound pustule formation not only in rosacea, but also in acne vulgaris and in some cases of perioral dermatitis (Baran & Civatte, 1971; Sneddon, 1972; Weber, 1972).

Hydrocostisone does not have the same effect and has been used as a substitute for fluorinated steroids in the management of rosacea and perioral dermatitis. Applications of hydrocortisone do not delay the gradual disappearance of telangiectasis and the presumed reformation of dermal collagen. It has also been noted that when tetracycline is used to control pustulation in rosacea or perioral dermatitis, continuation of the fluorinated steroid prevents this improvement (Sneddon, 1972; Weber, 1972), whereas hydrocortisone has no such action.

In an attempt to determine whether the difference between hydrocortisone and the fluorinated steroids depended on the increased penetration and powerful effect of the latter or the presence of the fluorine atom, it was decided to investigate the effect of a powerful non-fluorinated steroid, hydrocortisone butyrate, on patients who showed facial steroid atrophy. 0·1% hydrocortisone butyrate cream has been shown to be as effective as 0·1% triamcinolone acetonide and superior to 1% hydrocortisone in the treatment of psoriasis under occlusion (Polano *et al.*, 1970). It has proved superior to

0·1% triamcinolone acetonide and 1% hydrocortisone in the treatment of eczema (Polano & Kanaar, 1973) and equal to fluocinolone acetonide in eczema and psoriasis (Ashurst, 1972).

METHOD

Twenty-eight patients were selected. Fifteen were suffering from rosacea which had been treated for long periods with topical fluorinated steroids and showed extensive telangiectasia and superficial atrophy; three were patients with severe rosacea who had used fluorinated steroids; there were two with atopic dermatitis who had a similar atrophic telangiectasia, and eight with perioral dermatitis who also had steroid induced telangiectasia as a prominent feature.

The patients were given 30 g of hydrocortisone alcohol 1% cream and 30 g of 0·1% hydrocortisone butyrate in the same base, in paired numbered tubes marked Left and Right, and instructed to apply the creams twice daily to the appropriate side of the face, being careful to wash their hands between treatments. The tubes were issued by the hospital pharmacy and the key to the contents of the tubes was retained by the manufacturers. In addition, the majority of patients were prescribed oxytetracycline 250 mg twice daily, as only severe cases were selected and it was considered that systemic treatment was necessary to control the underlying disorder.

The patients were re-examined by the same physician at weekly intervals until one side of the face appeared to have improved more than the other or, in those where no difference was detected, three months had passed. When a decision on improvement had been made local treatment was discontinued and the patient re-examined a week later, as it was considered important to discover if rebound pustulation occurred as it did when fluorinated steroids had been withdrawn.

RESULTS

The findings can be seen in Table 1. A difference between the two sides of the face could be detected in as short a time as 2 weeks, though in the majority it was 4 weeks or more.

In fifteen patients hydrocortisone butyrate gave better results, in six hydrocortisone was superior, and in seven no difference could be detected. Leaving out the equals ($\chi^2 = 3{\cdot}86$, $P = 0{\cdot}050$), which is significant. Cases 7 and 14, which had received only hydrocortisone before the trial started, improved more quickly with hydrocortisone butyrate. Patients 5, 16, and 20 showed a moderate rebound of the eruption after withdrawal of topical treatment, in each case on the hydrocortisone butyrate treated side. In patient 11 the hydrocortisone treated side relapsed.

DISCUSSION

This blind trial of hydrocortisone and hydrocortisone butyrate was performed to determine whether the more powerful steroid, hydrocortisone butyrate, would have the same adverse effects that the fluorinated steroids produce, namely telangiectasis, atrophy and rebound pustulation. In the trial which was carried out on patients who already showed steroid induced atrophy or perioral dermatitis the hydrocortisone butyrate did not prevent the beneficial effect of tetracycline, and though four patients showed a mild withdrawal rebound eruption, hydrocortisone butyrate proved to be superior to hydrocortisone as judged by the disappearance of telangiectasia and of the actual eruption. One can conclude that the non-fluorinated steroid hydrocortisone butyrate is, like hydrocortisone, a safe application for the face, and that since it has not delayed recovery of steroid induced atrophy it is unlikely to cause it. Whether the fluoride radical is a significant factor in the production of collagen atrophy remains unknown.

TABLE I.

Case	Sex	Age	Diagnosis	Duration	Previous treatment	Oxytetracycline	Result and time of difference
1	F	25	Perioral dermatitis	1 year	Betamethazone and fluocinolone	Yes	H. 3 weeks
2	M	55	Rosacea with atrophy	3 years	Betamethazone	Yes	L. 4 weeks
3	M	29	Rosacea with atrophy	3 years	Fluocinolone	Yes	L. 4 weeks
4	F	35	Rosacea with atrophy	6 years	Betamethazone	No	Equal
5	M	38	Rosacea with atrophy	4 years	Betamethazone	Yes	L. 4 weeks*
6	F	58	Rosacea with atrophy	7 months	Betamethazone	Yes	L. 2 weeks
7	M	56	Rosacea	6 months	Hydrocortisone	Yes	L. 3 weeks
8	F	27	Perioral dermatitis	2 years	Betamethazone	Yes	Equal
9	F	29	Rosacea with atrophy	6 years	Betamethazone	Yes	H. 2 weeks
10	F	33	Perioral dermatitis	3 months	Betamethazone and fluocinolone	No	Equal
11	F	42	Rosacea with atrophy	15 years	Betamethazone	Yes	Equal*
12	F	31	Perioral dermatitis	3 months	Fluocinolone	Yes	L. 5 weeks
13	F	21	Perioral dermatitis	1 year	Betamethazone	Yes	L. 2 weeks
14	F	27	Rosacea	8 months	Hydrocortisone	Yes	L. 10 weeks
15	M	48	Rosacea with atrophy	6 months	Fluocinolone	Yes	L. 4 weeks
16	F	29	Perioral dermatitis	6 months	Betamethazone	No	L. 4 weeks*
17	M	24	Atopic dermatitis and atrophy	3 years	Betamethazone	No	L. 4 weeks
18	F	43	Rosacea with atrophy	2 years	Betamethazone	Yes	H. 10 weeks
19	M	52	Atopic dermatitis and atrophy	5 years	Betamethazone	No	H. 9 weeks
20	F	54	Perioral dermatitis	1 year	Fluocinolone	Yes	L. 3 weeks*
21	M	39	Rosacea with atrophy	6 years	Fluocinolone	Yes	Equal
22	F	20	Perioral dermatitis	7 months	Betamethazone	Yes	H. 4 weeks
23	F	46	Rosacea with atrophy	5 years	Betamethazone	Yes	L. 3 weeks
24	F	47	Rosacea	9 months	Betamethazone	Yes	Equal
25	F	33	Rosacea with atrophy	3 years	Betamethazone	Yes	Equal
26	F	58	Rosacea with atrophy	10 years	Betamethazone	Yes	H. 5 weeks
27	F	24	Rosacea with atrophy	4 years	Betamethazone	Yes	L. 3 weeks
28	M	49	Rosacea with atrophy	5 years	Betamethazone	Yes	L. 2 weeks
							15 L., 6H., 7 equal

L., hydrocortisone butyrate. H., hydrocortisone. * Withdrawal rebound.

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Human models for *in vivo* and *in vitro* assessment of topical antifungal compounds

ARTHUR G. KNIGHT

Division of Dermatology, Scripps Clinic and Research Foundation, La Jolla, California 92037, U.S.A.

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SUMMARY

Topical antifungal agents, 1% haloprogin solution (Halotex®), 1% tolnaftate solution (Tinactin®), 1% sodium omadine cream and 5% thiabendazole in dimethylacetamide have been investigated. They were tested for prophylactic effect against experimental human *Trichophyton mentagrophytes* infections. All the drugs were totally effective against experimental infection if the inoculation was immediate. However, after 48 h the effects of haloprogin and tolnaftate were greatly reduced, but the others were still very effective.

Stratum corneum may be stripped onto a tape, sterilized and then inoculated with *T. mentagrophytes* spores. Under controlled conditions the spores germinate *in vitro* in the stratum corneum. This method has been quantitated and adapted to the investigation of topical antifungal agents. Results show that tolnaftate has very little antifungal activity even at zero time, thiabendazole and sodium omadine retain excellent activity in the stratum corneum for 48 h and are readily detectable even 72 h after one application.

The assessment of topical antifungal compounds has been fraught with difficulties, the main problem being that *in vitro* testing usually bears little resemblance to clinical usage. The growth of a dermatophyte on a nutrient culture medium is not comparable to its growth on stratum corneum *in vivo* or *in vitro* (Nannizzi, 1926; Emmons, 1934; Klein, 1965). Clinical trials are difficult to control because of the lack of uniformity of naturally occurring lesions.

With these problems in mind two new techniques have been utilized and adapted to the study of antifungal compounds. The first is the use of standard reproducible experimental *T. mentagrophytes* infections in humans (Knight, 1972a), and the other an *in vitro* system for the culture of dermatophytes on stratum corneum (Knight, 1972b).

Other workers have utilized experimental infections with dermatophytes to investigate the therapeutic and prophylactic effects of systemic griseofulvin (Esteves & Neves, 1959; Desai & Bhat, 1960; González-Ochoa, Ricoy & Bravo-Becherelle, 1964). More recently, human experimental infections with *T. rubrum* were used to evaluate the therapeutic effect of topical haloprogin (Hermann, 1972). In the past when using *T. mentagrophytes* infections the author has found difficulty in detecting therapeutic effect because of the natural remission of the infections. For this reason it was decided initially to investigate the prophylactic effect of topical antifungal compounds against *T. mentagrophytes* experimental infections in humans.

It is possible to assay antifungal activity within the stratum corneum by culturing *T. mentagrophytes* spores on stripped stratum corneum. Growth of the dermatophyte may be compared on untreated and previously treated normal stratum corneum. By taking strippings over a period of time after one application of the drug, it is possible to follow the length of time that its activity remains in the stratum corneum.

MATERIALS AND METHODS

The same antifungal compounds were used in the *in vivo* and *in vitro* experiments. The drugs used were 1% haloprogin solution (Halotex), 1% tolnaftate solution (Tinactin), 1% sodium omadine cream, and 5% thiabendazole in dimethylacetamide (DMAC).

Haloprogin is a 3-iodo-2-propynyl 2,4,5-trichlorophenyl ether. It is a white or pale yellow crystalline compound which is only slightly soluble in water. Halotex 1% solution is solubilized in diethyl sebacetate and ethyl alcohol.

Tolnaftate is *o*-2-naphthyl-*m*-N-dimethylthiocarbanilate. The 1% solution is made up in polyethylene glycol 400.

Sodium omadine is a 1-hydroxypyridine-2-thione derivative. It is readily water soluble.

Thiabendazole is 2-thiazol-4-ylbenzimidazole. It is a white crystalline compound only slightly soluble in water.

For two weeks prior to and during all the experiments the subjects used Ivory soap which contains no antiseptic additives.

Experimental human T. mentagrophytes *infections*

Thirty subjects were utilized. All were skin tested by intradermal injections of Hollister-Stier Dermatophytin 1:30. The infections were induced by application of 10,000 spores in 0·05 ml of sterile water to the upper arm. The spore solution was covered by damp gauze. The surrounding area had been previously sprayed with Dow Corning Medical B spray adhesive. The whole area was then covered with a square of polythene pressed over the adhesive to achieve a seal.

Four inoculations were made on each upper arm. One arm acted as the control. The treated arm had four areas marked off—each 4 cm square and 5 cm apart. To each a different antifungal compound was carefully applied. This was left in place for 15 min. The sites were dabbed dry. They were then washed with a standard procedure of 10 s under running tap water and three strokes with Ivory soap and allowed to dry. The sites were randomly rotated.

Inoculations with *T. mentagrophytes* spores were made on the control and treated sites either immediately after drying, after a delay of 24 h of normal activity, or after 48 h of normal activity. The sites were occluded for 72 h. The dressings were removed and the sites examined 72 h later. Culture and microscopic examination for dermatophytes were made daily for 7 days. A positive infection was one which was either culturally or microscopically positive.

In vitro *assay for antifungal compounds in stratum corneum*

Stratum corneum was stripped off onto a piece of translucent adhesive tape (Steri-Drape) by repeated application directly to the skin. The tape is manufactured by Minnesota Mining & Manufacturing Company (St Paul, Minnesota) and has an inert copolymer as the adhesive. The tape was then placed, stratum corneum surface uppermost, onto a microscope slide and fixed at each end by pieces of the adhesive tape. The slide was placed on a bent glass rod in a petri dish and covered by a lid. The dish was sterilized in ethylene oxide gas for 4 h at 53°C at a pressure of 8 lb/in^2. 48 h were allowed for the gas to disperse. 8 ml of sterile water were run into the bottom of the dish and 0·005 ml of a spore suspension of *T. mentagrophytes* was applied to the centre of the tape and spread. The lid was replaced and the dish was stored at 30°C. After 7 days the area of inoculation was covered with a net-like mass of hyphae. If the fungus was growing under less than optimal conditions or if the stratum corneum contained an antifungal compound, the hyphae tended to be shorter. If the stratum corneum contained a high concentration of a fungistatic agent, only small, swollen, germinal tubes were produced.

Grading of growth

Cultures were incubated for 7 days before grading. A grading system of 0–3 was adopted. Grade 0 was no germination or the production of short fat hyphae which remained within the scales of stratum corneum. Grade 1 was the production of hyphae which tended to branch mainly within the stratum corneum scale but also to cross over areas of tape to nearby scales. Grade 2 was the most common one on normal stratum corneum. There were long straight hyphal filaments traversing numerous stratum corneum scales. Grade 3 was the same situation as Grade 2 with an additional feature of distinct thalli growing on the stratum corneum scales.

Adaptation of the method to the study of topical antifungal agents

The upper arms were used as test sites. Strippings were taken prior to the application of an antifungal compound. Five areas 9 × 5 cm were marked out on the upper arms. The drug was applied to the entire area within each rectangle and left for 15 min. All the areas were then given a standardized wash of 10 s under running tap water with three strokes of Ivory soap. The areas were dabbed dry and strippings were taken from area 1 at zero time, from area 2 after 8 h of normal activity, area 3 after 24 h, area 4 after 48 h, and area 4 after 72 h. The sites were in no way protected and each subject bathed once daily using Ivory soap. The strippings were sterilized, inoculated and cultured as detailed above. After 7 days incubation they were examined and a grading of 0–3 was given. All gradings were observed in a double-blind fashion.

RESULTS

The results of the experimental infections are summarized in Table 1. Only five subjects had positive delayed responses to trichophyton antigen, but this did not appear to affect experimental results. When the spores were applied immediately after washing the treated sites, only one infection resulted, that being on a site treated with haloprogin. All others were negative and all control sites were positive. After a delay of 24 h of normal activity and then application of the spores, 1% sodium omadine cream was still completely effective as a prophylactic. Only one infection resulted on the 5% thiabendazole in DMAC treated sites. The 1% haloprogin and 1% tolnaftate solutions were less effective, three and five infections resulting out of a possible ten. Two of the control sites failed to produce positive infections due to the dressings becoming loose. After 48 h, sodium omadine and thiabendazole were still very effective, one and three infections resulting respectively. Haloprogin and tolnaftate were less effective, six and seven infections respectively out of a possible ten. Analysis by Student's '*t*' test is shown in Table 1.

Results of the *in vitro* technique for detection of antifungal compounds in the stratum corneum are summarized in Table 2. The numbers indicating the degrees of growth are the sums of the gradings of growth from twelve tape strippings, three from each of four subjects. With a maximum grading of 3, each has a maximum score of 3 × 12 = 36. All pretreatment scores approximate the maximum score. At zero time the antifungal activity within the stratum corneum, after treatment with haloprogin,

TABLE 1. Results of human experimental *T. mentagrophytes* infections after prophylactic use of topical antifungal agents

Time	Number of positive infections				
	Normal sites	Treated sites			
		Haloprogin	Tolnaftate	Sodium omadine	Thiabendazole
0 h	40/40	1/10	0/10	0/10	0/10
Probability		$P<0\cdot01$	$P<0\cdot01$	$P<0\cdot01$	$P<0\cdot01$
24 h	38/40	3/10	5/10	0/10	1/10
Probability		$P<0\cdot01$	$P<0\cdot05$	$P<0\cdot01$	$P<0\cdot01$
48 h	38/40	6/10	7/10	1/10	3/10
Probability		NS	NS	$P<0\cdot01$	$P<0\cdot01$

There was a significant difference between sodium omadine and tolnaftate at both 24 and 48 h ($P<0\cdot01$), and between sodium omadine and haloprogin at 48 h ($P<0\cdot02$).

Probability assessed by Student's '*t*' test.

NS, not significant.

TABLE 2. A comparison of the effectiveness of topical antifungal compounds as shown by inhibition of *T. mentagrophytes* spore growth on stratum corneum *in vitro*

Time	Degree of growth			
	Haloprogin	Tolnaftate	Sodium omadine	Thiabendazole
Pretreatment	34	32	34	34
0 h	2	30	0	0
Probability	$P<0\cdot01$	NS	$P<0\cdot01$	$P<0\cdot01$
8 h	14	28	0	0
Probability	$P<0\cdot05$	NS	$P<0\cdot01$	$P<0\cdot01$
24 h	32	30	13	2
Probability	NS	NS	$P<0\cdot05$	$P<0\cdot01$
48 h	33	33	13	16
Probability	NS	NS	$P<0\cdot05$	$P<0\cdot05$
72 h	32	35	17	23
Probability	NS	NS	NS	NS

Haloprogin and tolnaftate were less effective than thiabendazole and sodium omadine after 8 h ($P<0\cdot01$).

Probability assessed by Student's '*t*' test.

NS, not significant.

sodium omadine, and thiabendazole was sufficient to completely inhibit hyphal production. However, no significant antifungal activity was detected in the superficial stratum corneum immediately after therapy with tolnaftate, and none was detected thereafter. 8 h later, sodium omadine and thiabendazole were still completely effective, but much of the effect of haloprogin was lost. After 24 h of normal activity, strippings taken showed that the thiabendazole was still very effective, the haloprogin had lost its effect, and sodium omadine was intermediate between the two in effect. At 48 and 72 h both sodium omadine and thiabendazole still showed antifungal activity in the stratum corneum. Analysis by the '*t*' test is shown in Table 2.

The cream base of the sodium omadine and the DMAC were also tested for activity against *T. mentagrophytes* spores with this technique, and neither showed any activity.

DISCUSSION

Both of these experimental models showed that the preparations of sodium omadine and thiabendazole were the most effective. Both drugs had a marked inhibitory effect 48 h after one application and were readily detectable in the stratum corneum *in vitro* for at least 72 h. Both drugs penetrate through stratum corneum *in vitro* (R.B. Stoughton, personal communication). Sodium omadine has been marketed as a 1% cream in France for some years, and an uncontrolled clinical trial in 1969 (Risold & Pequegnot, 1969) showed it to be very effective against a broad spectrum of fungus disease. However, it was found to be irritating when applied to highly inflamed lesions. Thiabendazole was first introduced as a broad spectrum anthelmintic in animals and was later approved for use in man. Subsequently, it was found to possess antimycotic activity. *In vitro* studies (Robinson, Phares & Graessle, 1964) showed it to be very active against a wide range of fungi, and to be very effective against dermatophytes. Clinical trials have shown its effectiveness (Fleischmajer, Goldstein & Nicholas, 1965; Thomas, Saint-André & Cornudet, 1971).

The results with the two models using haloprogin were also comparable, and by 24 h it was shown to have lost most of its prophylactic effect. It has been found to be effective in the treatment of experimentally induced topical mycotic infections in guinea-pigs by Harrison *et al.* (1970). In a further study 1% haloprogin cream was reported to be more effective than the placebo ($P < 0{\cdot}05$) in the treatment of human experimentally induced *T. rubrum* groin infections (Hermann, 1972).

The results with tolnaftate are different for the two models. By the experimental infection method, no infections resulted when the spores were applied immediately following tolnaftate application. However, no inhibition was detected with the *in vitro* model. It seems likely that, with the *in vivo* experiments, the more potent antifungal compounds may have spread locally onto the tolnaftate sites. The *in vitro* experiments were performed using only one drug at a time and so contamination of different sites was not a problem. Weinstein, Oden & Moss (1964), using the guinea-pig as an experimental model and *T. mentagrophytes* as the infecting organism, found that a tolnaftate effect was demonstrable 10 days after two applications given on the same day. Robinson & Raskin (1964) in a clinical trial showed that the 1% solution was effective in the treatment of superficial mycotic infections.

The two techniques, using human models, are relatively simple and produce reasonably consistent results. They indicate that topical antifungal agents for prophylaxis are effective, and that one brief application may give protection lasting over 72 h. With the exception of tolnaftate, the stratum corneum technique results indicate the same degree of prophylaxis with each agent as found with these *in vivo* human models. This may be helpful in future assays because studies with the stratum corneum dermatophyte culture model are considerably easier to perform than are those using *in vivo* human experimental infections.

One could speculate that the differences found with the various antifungal preparations in regard to prophylaxis would also hold for the treatment of active dermatophyte infections in man. It is intended to conduct relevant studies in the near future.

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The therapeutic value of retinoic acid in chronic discoid, acute guttate, and erythrodermic psoriasis: clinical observations on twenty-five patients

S. GÜNTHER

Department of Dermatology, University of Glasgow

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SUMMARY

Twenty-five patients with chronic discoid, acute guttate or erythrodermic psoriasis were treated with retinoic acid in various preparations, topical and oral, and the clinical response was observed for up to 3 months. Only four patients showed distinct clinical improvement. The possible side effects of topical retinoic acid therapy are mentioned.

Several papers published during the last few years have drawn attention to the benefit of retinoic acid in chronic skin conditions, as compared with placebos. In psoriasis the therapeutic value of retinoic acid seems to be uncertain: the investigations of Beer (1962), Frost & Weinstein (1968, 1969), MacDonald, McMinn & Fry (1972), and MacDonald & Fry (1972), have produced conflicting results. The object of this study is to attempt to prove or disprove the effectiveness of retinoic acid in different forms of psoriasis.

PATIENTS AND METHODS

Twenty-five patients, nineteen male and six female, age range 14–76 years, suffering from different forms of psoriasis were chosen for the trial: chronic discoid psoriasis (sixteen cases), acute guttate psoriasis (seven cases), and erythrodermic psoriasis (two cases).

In seventeen patients, twelve male and five female, the topical administration of retinoic acid 0·1% in white petroleum jelly base was used daily, or every second day, with or without polythene occlusion. A controlled unilateral trial with the active preparation versus a placebo was carried out in twelve cases. All the patients were examined weekly, over a period up to 3 months, and the clinical events were noted.

Eight patients, seven male and one female, were treated systemically with retinoic acid, 16·0 mg or 12·5 mg in hard gelatine capsules, which were taken 1 h before meals, once or twice daily.

All the preparations of retinoic acid were kept in a refrigerator because of its instability. Liver function tests, transaminase estimations, and blood examinations were carried out before, during, and at the end of therapy. Patients with abnormal liver function tests and pregnant mothers were excluded from the trial.

RESULTS

The results of these investigations in different forms of psoriasis are summarized in Table 1.

Of the sixteen patients with chronic discoid psoriasis, twelve were treated with topically applied retinoic acid: in ten, doubtful or no improvement was observed; only two patients with severe hyperkeratotic plaques showed distinct involution, as compared with those treated with the placebo. In four patients, to whom retinoic acid was administered systemically, the lesions remained unchanged during the time of observation.

TABLE 1. Twenty-five patients with various forms of psoriasis treated with retinoic acid by different routes of administration

Diagnosis	No. of cases	Topical treatment	Systemic treatment	Clinical improvement within up to 3 months of observation	
Chronic discoid psoriasis	12	Up to 10 weeks	—	+*	2 cases
				o†	10 cases
Chronic discoid psoriasis	4	—	Up to 2 weeks	+	None
				o	4 cases
Acute guttate psoriasis	3	Up to 4 weeks	—	+	1 case
				o	2 cases
Acute guttate psoriasis	4	—	Up to 3 weeks	+	1 case
				o	3 cases
Erythrodermic psoriasis	1	1 week	—	+	None
				o	1 case
Erythrodermic psoriasis	1	—	1 week	+	None
				o	1 case

*+, distinct improvement.
†o, doubtful or no improvement.

In a group of seven patients suffering from acute guttate psoriasis, two young boys improved after 1 week of topical or systemic retinoic acid therapy. Two patients with erythrodermic psoriasis failed to respond to either local or systemic retinoic acid administration.

Only three of ten patients tolerated the 0·1% retinoic acid ointment when it was applied daily. Diffuse erythema of the treated areas developed in nine patients, one of whom showed also inflammation of the scrotum. Folliculitis with pruritus appeared in one patient using retinoic acid under polythene occlusion.

In all the patients the results of the laboratory tests remained within normal limits.

DISCUSSION

This small survey suggests that retinoic acid, topically or systemically administered, seems in general to be of little therapeutic value on psoriatic skin, and successful only in marked by hyperkeratotic lesions. After daily topical applications under polythene occlusion, unacceptable irritant reactions, with folliculitis, often become troublesome.

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Case Reports

Juvenile pemphigoid

W.R.FABER AND TH.VAN JOOST

Department of Dermatology (Chairman: Professor Dr R. H. Cormane), University of Amsterdam, Binnengasthuis, The Netherlands

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The aim of this communication is to discuss some aspects of the classification of juvenile dermatitis herpetiformis on the basis of a case report.

CASE REPORT

A 6-year-old boy was sent for consultation to our clinic with a history of bullae on the scalp, on the face, around the nose, behind the ears, followed by lesions on the scrotum and in the inguinal regions. His health was not impaired, and apparently the lesions were not pruritic.

On first examination no bullae could be detected on the skin. However, a fortnight later a group of bullae developed on the left side of the scrotum and in the left inguinal region. Some days later a few bullae appeared around the nasal orifices and on the upper lip, followed by a few solitary bullae on the trunk and extremities. All bullae appeared on normal skin or on a slightly erythematous base; their mean diameter was 0·5–1 cm. The patient received no treatment at the time of investigation. Later he was treated with sulphapyridine with satisfactory results.

Laboratory results

ESR 12 mm in 1 h; Hb 6,8 mmol/l; WBC 6500; eosinophils 3%; red blood cells, mild hypochromia. Haptoglobin, normal. Total serum proteins, normal. Serum electrophoresis, normal. Immunoelectrophoresis, IgA slightly decreased. Urine: no albumin sugar, or abnormal deposits. Faeces: normal colour and consistency. Glucose tolerance curve, normal.

Iodide patch test

Tincture of iodine 1%; bullous reaction after 24 h. Potassium iodide 25% in soft paraffin; vesico-bullous reaction after 24 h. Potassium iodide 50% in soft paraffin; vesico-bullous reaction after 24 h. These patch tests were not biopsied.

Histological report

There is a subepidermal bulla, the roof of which is intact, containing serous exudate and relatively few inflammatory cells. The surrounding dermal region exhibits oedema and only sparse inflammatory infiltration. No conspicuous papillary micro-abscesses are seen. Conclusion: a subepidermal bulla; the histopathological findings are not diagnostic.

Immunofluorescence studies

Conjugate	Batch no.	F/P ratio (mol × 10^{-3})	Protein content (mg/ml)	Working dilutions
Characteristics of conjugates used for direct tests				
RAHuIgG/FTC*	0170	1·10 2·5	10	1 : 20
GAHuIgA/FTC*	6-171	0·94 2·4	17·8	1 : 32
GAHuIgM/FTC*	7-171	1·25 3·0	16·4	1 : 32
RAHuFbg/FTC*	1170	4·14 4·14	11·2	1 : 32
Sera and conjugates used for indirect tests				
SwAHuIgD†	K5			1 : 32
RAHucompl.†	KH-13-16-AL			1 : 32
GAR/FTC*	3-171	1·20 2·6	13·7	1 : 64
RASw/FTC*	2969	0·9 8·1	1·76	1 : 32

These data were provided by the purchasers: *Nordic Pharmaceuticals and Diagnostics, Tilburg, Holland; † The Netherlands Red Cross Blood Transfusion Service, Amsterdam, Holland.

A Leitz Ortholux fluorescence microscope equipped with epi-illumination was used. Exciting filters BG 38 (4 mm), KP 490 (1 mm) 2 × in combination with trimmer filter EK 470 for blue narrow band excitation and a barrier filter EK 515 (8 mm) were used.

Biopsies were taken from perilesional and unaffected skin for immunofluorescence studies. The procedures have been described previously.

Direct immunofluorescence

	Perilesional skin		Unaffected skin (right forearm)	
IgG	++	Predominantly linear, partly granular	+	Predominantly linear, partly granular
IgA	+++	Linear	+++	Linear
IgM	–		–	
IgD	–		–	
Fibrinogen	–		–	
Complement	+++	Predominantly linear, partly granular	++	Predominantly linear, partly granular

+++, strong fluorescence; ++, moderate fluorescence; +, weak fluorescence; –, no fluorescence.

Indirect immunofluorescence. (a) No circulating antibodies directed against basement membrane could be detected, using various substrates, namely normal human skin, human oral mucosa, human intestinal mucosa, and guinea-pig lip. (b) No antibodies directed against basement membrane could be detected in the blister fluid, using normal human skin and guinea-pig lip as substrate. (c) No ANA and auto-antibodies against erythrocytes, mitochondria, smooth muscle, skeletal muscle, adrenal gland, thyroid gland (colloid and cytoplasma) or gastric parietal cells could be detected.

DISCUSSION

It is generally assumed that in infants and children under 10 years old dermatitis herpetiformis has a distinctive clinical appearance (Kim & Winkelmann, 1961; Ganpule, 1967; Grant, 1968). Our case fits in with the above mentioned group.

The results of the immunofluorescence studies in this case are comparable with those of two (nos. 6 and 7) out of eight cases with juvenile dermatitis herpetiformis reported by Jablonska *et al.* (1971).

IgG, IgA and complement appear to be concomitantly present in the junctional zone of the perilesional skin and the unaffected skin (Fig. 1). The pattern of fluorescence was of a mixed type, i.e. linear and granular, as has been described in two out of five pemphigoid cases by Cormane & Szabo (1970). In a later publication Cormane & Gianetti (1971) reported the concomitant *in vivo* occurrence of IgG, IgA, IgM, IgD and complement in a linear staining pattern in one out of five pemphigoid cases.

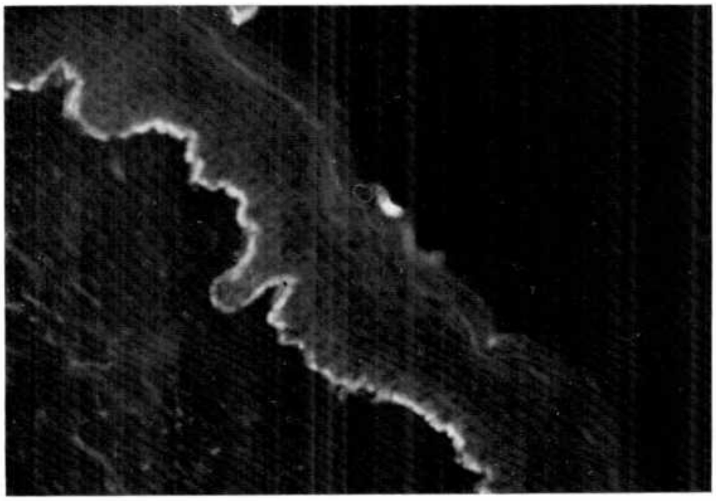

FIGURE 1. Direct immunofluorescence staining with anti-IgA conjugate of biopsy of peribullous skin. Note a linear fluorescence of the basement membrane zone (× 220).

Papillary deposition of fibrinogen, a characteristic of dermatitis herpetiformis was not present in our case (Mustakallio, Blomqvist & Laiho, 1970).

No circulating antibodies were detectable using various tissues as substrate. In this respect this case differs from the first case described by Bean, Good & Windhorst (1970) in which a high titre (1 : 1280) of circulating pemphigoid antibodies could be demonstrated.

The presence of IgG and complement in the upper layers of the dermis has been described by Fincher, Dupree & Bean (1971) in a case which they classified as bullous pemphigoid in childhood since circulating antibodies against basement membrane were present.

In conclusion, it can be said that in our case, the immunofluorescence findings correspond to a great degree with those found in bullous pemphigoid in adults, but without circulating antibodies.

In general, it can be said that classifications based on clinical as well as laboratory findings are hazardous as long as the aetiology of the disease is unknown.

With regard to the nomenclature, the term juvenile pemphigoid, as used for example by Sneddon (1972), is in our opinion to be preferred for such cases.

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Disseminated perforating granuloma annulare

T.J.DELANEY, S.C.GOLD AND BARBARA LEPPARD*

Department of Dermatology, St George's Hospital, London, S.W.1

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SUMMARY

A case of generalized granuloma annulare is presented in which many of the lesions showed the phenomenon of epidermal perforation.

Granuloma annulare, a relatively common dermatosis, usually presents with a characteristic and easily recognizable clinical picture. The majority of lesions occur as discrete ringed eruptions on the limbs (Wells & Smith, 1963). However, atypical forms are well recognized, including giant forms and a subcutaneous nodular variety (Beare & Wilson Jones, 1972). Disseminated, or generalized granuloma annulare is a rare variety (Dicken, Carrington & Winkelmann, 1969), which in some patients may be restricted to sun exposed areas of skin (Leppard & Black, 1972). A perforating type has also been described (Calnan, 1954; Owens & Freeman, 1971), the lesions in these patients being few and confined to the extremities.

A case is presented of disseminated granuloma annulare in which many lesions also showed epidermal perforation with release of necrobiotic material.

CASE REPORT†

A 60-year-old Indian ship's captain presented in April 1972 with a 12-year history of non-irritant skin lesions. In 1959, routine urine examination had shown glycosuria, diabetes being subsequently confirmed by a glucose tolerance test. He had been treated with tolbutamide for 3 weeks, but subsequently by diet alone. There was no family history of skin disease, but his father was diabetic.

He developed one small spot on his left arm in 1960. Biopsy was performed, a diagnosis of tuberculide made, and he was treated with PAS and isoniazid for 6 months. During this time many more spots appeared, some of which showed a cycle of development. Lesions have continued to appear at varying rates since then, involving most areas of his skin.

The majority of the lesions progress through a definite series of stages. A red papule appears, enlarges and then develops a yellowish centre. On gentle squeezing, or spontaneously, the centre then discharges a little clear viscous fluid, which dries to form a crust. The crust eventually becomes detached leaving a small hyperpigmented scar. The cycle for each individual lesion lasts 2–3 months. Some red papules do not discharge but regress to leave no apparent mark.

Physical examination showed a remarkable appearance. The rash involved most areas of skin except for his palms, soles and face, with maximum involvement of the anterior thigh and calf (Fig. 1). Lesions in various stages of development were discernible. The earliest lesions appeared as 2–3 mm erythematous papules. Slightly later lesions had a yellowish central zone resembling a pustule (Fig. 2), from some of which a little fluid could be expressed. Later, ulcerated lesions developed a moist crust with a collarette of scale. Old lesions were represented by a pigmented slightly depressed scar.

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* Present address St John's Hospital for the Diseases of the Skin, Lisle Street, London, W.C.2.

† Shown at the clinical meeting of the St John's Hospital Dermatological Society on 29 June 1972.

General examination was normal apart from the presence of arcus senilis. There was no evidence of diabetic retinopathy.

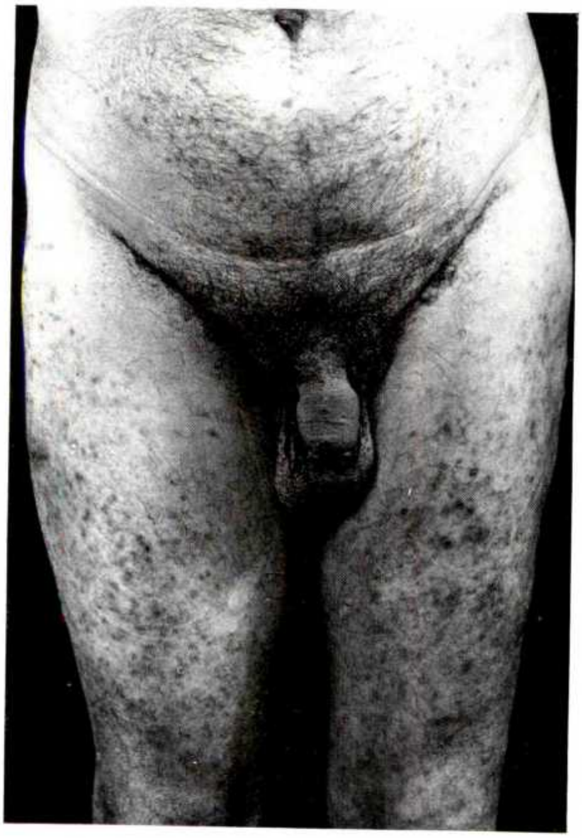

FIGURE 1. Disseminated perforating granuloma annulare. Appearance of rash on thighs.

Biopsies were taken of lesions in various stages of development.

(a) From a lesion with a yellowish central zone (corresponding to lesion A in Fig. 2). This showed a circumscribed focus of collagen degeneration in the dermis surrounded by an infiltrate composed of histiocytes and lymphocytes. The appearances were consistent with the diagnosis of granuloma annulare.

(b) From a perforating lesion (corresponding to lesion B in Fig. 2). This showed (Fig. 3) a small rounded lesion in the dermis close to the epidermis, with central ulceration through the epidermis. The ulcerated area of the lesion contained pus cells. The periphery of the lesion showed palisading histiocytes together with a chronic inflammatory infiltrate.

(c) From an erythematous papule. This showed a perivascular infiltrate composed of macrophages

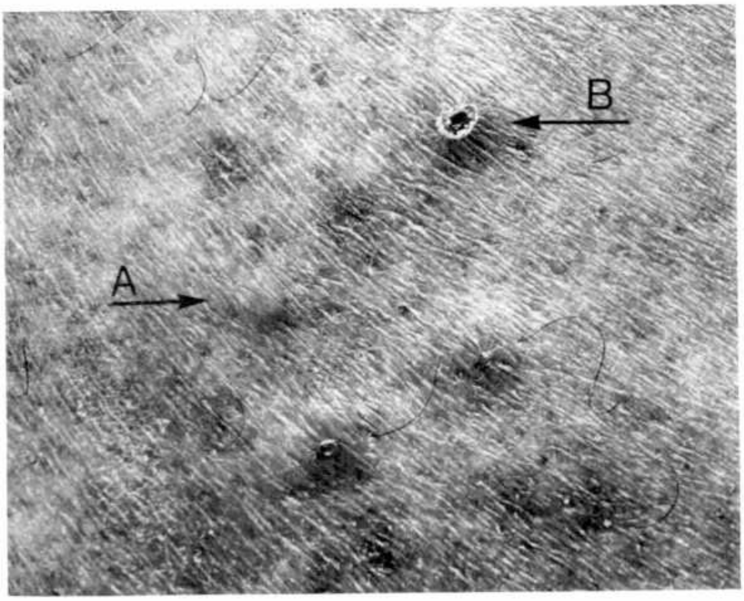

FIGURE 2. Disseminated perforating granuloma annulare showing lesions in various stages of development. A, early lesion with yellowish centre; B, perforating lesion with collarette of scale.

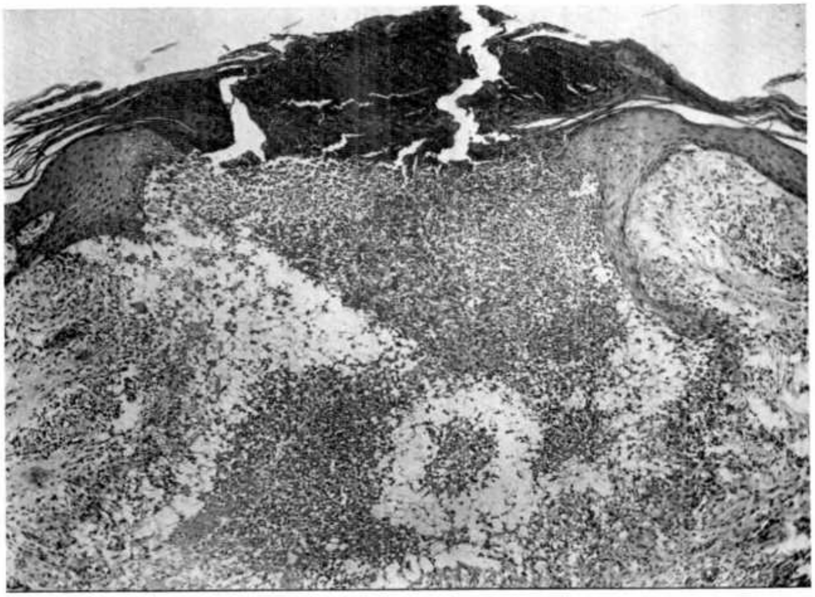

FIGURE 3. Disseminated perforating granuloma annulare (×35).

and lymphocytes situated in the superficial dermis. Associated with the infiltrate were foci of collagen degeneration.

(d) From an area of scarring. This showed dermal scarring and epidermal thickening, but no definite changes of granuloma annulare.

(e) From clinically normal skin. This showed one area of reduced elastica, but no other abnormality.

Routine investigations including blood count, ESR, serum cholesterol, serum proteins and chest X-ray were normal. Glucose tolerance test showed a diabetic curve. Lipoprotein estimation showed a slight increase in pre-β lipoprotein.

DISCUSSION

We believe that this case is one of widespread perforating granuloma annulare.

From the evidence presented here, it would appear that the mechanism of perforation was by ulceration of superficial lesions with subsequent healing and scar formation. This case does not appear to exhibit true 'transepithelial elimination' as described by Mehregan (1968), as in this phenomenon material is carried through the epidermis with little or no damage to the epithelium. However Mehregan's illustration of the histology of perforating granuloma annulare does show some resemblance to the picture in our patient.

Some of the papules in this patient do not perforate, but eventually involute spontaneously as in typical granuloma annulare. Perhaps, as suggested by Owens & Freeman (1971), it is simply the very superficial location of some foci of granuloma annulare which allows ulceration and discharge of necrobiotic material.

ACKNOWLEDGMENTS

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Squamous cell carcinoma of skin involving the median nerve

D.J.DANDY AND D.D.MUNRO

Orthopaedic and Dermatology Departments, St Bartholomew's Hospital, London, E.C.1,
and Edgware General Hospital, Middlesex

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SUMMARY

The case is described of a patient with a squamous cell carcinoma of the skin of a finger which spread in an unusual manner, caused median nerve compression at the wrist, and infiltrated the median nerve in the forearm.

CASE REPORT

A 51-year-old insurance agent noticed a red nodule on the skin of the dorsum of the proximal phalanx of his right middle finger. The nodule was tender if knocked but not painful. The patient was first examined 3 months after the lump appeared and a confident diagnosis of keratoacanthoma was made in view of the well circumscribed tumour with rolled edge and keratotic central plug. The lesion was treated by curettage and the base cauterized with electrocautery. The histology of the nodule showed proliferative epithelium consistent with the clinical diagnosis.

One week after the initial treatment, the wound had healed but the patient complained of pain in the finger, and 1 month later pain in the finger, hand and right elbow was his main complaint. By this time the site of the tumour had become indurated and erythematous with lymphangitis on the dorsum of the hand, and the appearance was suggestive of a chronic granuloma due to infection. The surface remained intact but the lesion did not resolve with antibiotic and symptomatic therapy, though the lymphangitis cleared.

The indurated area on the finger gradually increased in size and the patient noticed paraesthesia in the tip of the affected finger and continued to complain of pain in the hand.

Six months after the original onset, a second biopsy was taken which showed the appearances of a well differentiated squamous cell carcinoma with development of burrowing crypts lined by squamous epithelium and filled with keratin debris. Culture of some tissue from the specimen was undertaken to exclude chronic granulomatous infection but was sterile apart from a proteus organism. The lesion by this time was 1 cm in diameter, ulcerated at its centre and bore several firm raised and reddened areas at its edge.

The finger was amputated through the shaft of the metacarpal and the tendons divided well back in the palm. The skin incision was 3 cm proximal to the lesion at its closest point and excision appeared complete. Shortly after this operation the patient felt more troublesome pain in his index, thumb, and the radial border of his ring finger, which was worse after using his hand and at night. 8 months after amputation of the finger the patient fell onto his outstretched right hand. There was no evidence of a fracture either clinically or radiologically, but his pain became more severe and a firm, tender swelling about 1 cm wide and 3 cm long could be felt beneath the skin in the line of the median nerve above the wrist.

The median nerve was explored through a longitudinal incision and was found to be encased in a firm, greyish-white material which was quite free from the surrounding tissues. The abnormal tissue was separated from the nerve by a thin, yellow, turbid fluid for most of the length exposed, but was seen invading the nerve in an area about 5 mm in diameter. A specimen of the abnormal tissue was removed and the wound closed. Histological examination of the specimen showed fibrous tissue invaded by strands of a poorly differentiated squamous cell carcinoma.

The arm was amputated above the elbow and dissected before formalin fixation.

After the patient recovered from the amputation he remained well for 16 months and then developed pain in the spine due to metastatic deposits with collapse of the T10 vertebra. A chest radio-

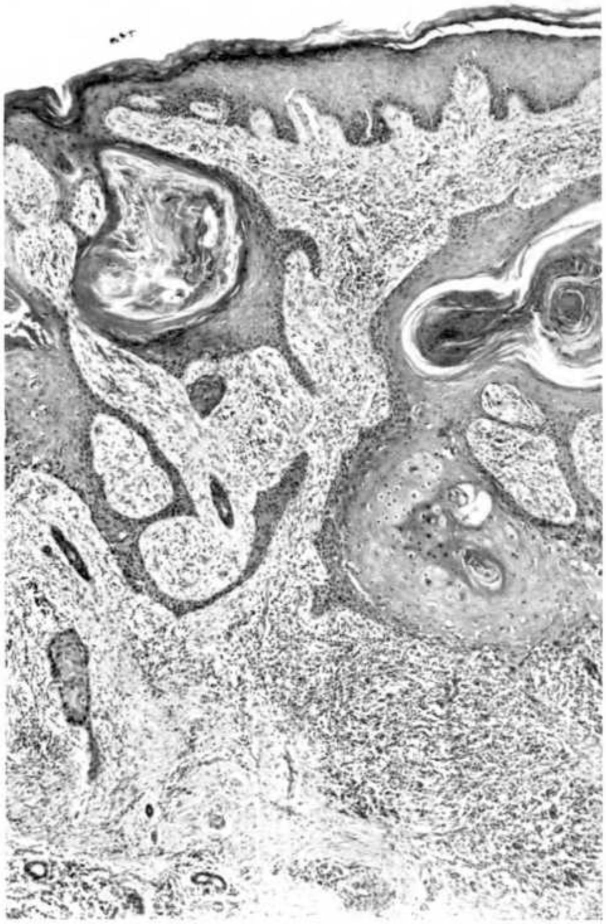

FIGURE 1. Sections of skin lesion. There is a proliferation of epithelial cells producing cords and nests which extend deep into the epidermis. The cells are hyperchromatic and pleomorphic. There is an associated inflammatory reaction (H & E, × 50).

graph showed cavitating metastases in the lungs. Despite radiotherapy the patient's condition rapidly deteriorated and he died 18 months after the arm amputation. No autopsy was held.

Findings at dissection

There was no sign of tumour on the skin of any part of the hand or arm. The median nerve was exposed from the site of amputation, 8 cm above the elbow, to the base of the fingers and was found to be surrounded by woody neoplastic tissue from a point 12 cm above the wrist joint to its division into branches. A terminal neuroma was found on the stump of the branch to the radial side of the middle finger, but there was no macroscopic sign of the tumour involving the cutaneous branches to the amputated middle finger. Except for the length of nerve explored surgically, the affected nerve separated easily from its bed. At the upper limit of the tumour there was gradual narrowing from the diseased nerve to normal nerve above. The median and ulnar nerves were removed and a careful search made for tumour elsewhere in the arm. The epitrochlear node was absent and no tumour could be found apart from that in the median nerve.

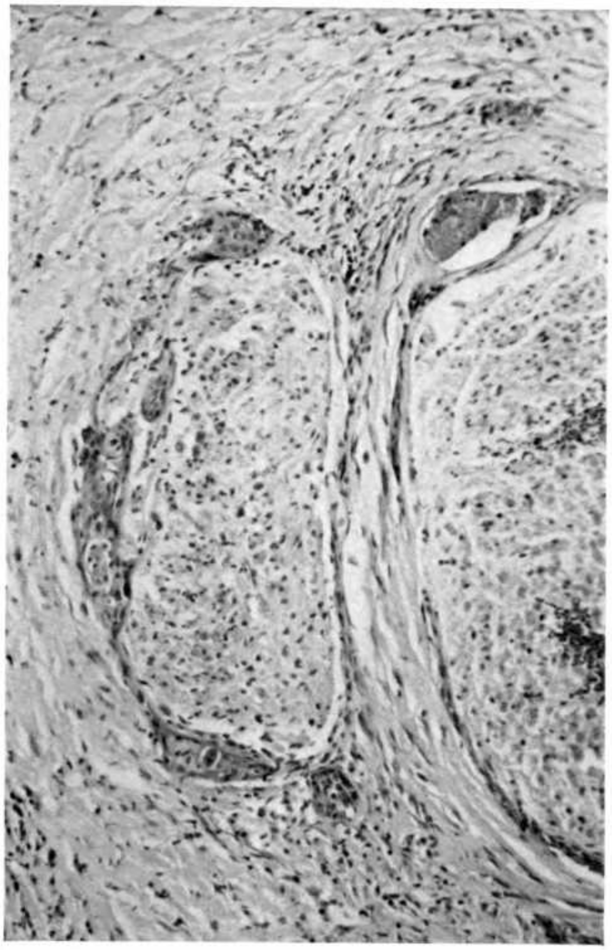

FIGURE 2. Portions of two fasciculi with tumour in the epineural lymphatics of the median nerve (H & E, × 320).

Pathological examination

Histological examination of sections of the median nerve showed areas of infiltration by well differentiated squamous cell carcinoma. In some areas the tumour was producing normal looking keratin which appeared as 'horn pearls' within the nerve.

DISCUSSION

It is well known that certain tumours can spread along nerves (Willis, 1952), although it is a matter of debate whether such spread occurs along perineural lymphatics or simply along a convenient tissue plane, or even whether perineural lymphatics exist at all (Dodd *et al.*, 1970). There are several reports of squamous cell carcinoma of the nasopharynx extending along the cranial nerves (Mardjono, Supeno & Markham, 1968; Lehrnbecher *et al.*, 1970; Thomas & Waltz, 1965), but in the few reports of squamous cell carcinoma of skin involving nerves (Dodd *et al.*, 1970; Vandooren-Deflorenne, Lachapelle & Bourland, 1967) most lesions arose on the face, usually on the lower lip or eyelid, though one involved the penis (Mohs, 1956). Most reports describe direct spread from the primary tumour along the adjacent nerve. No such direct spread was found in the patient described here.

Mohs (1956) noted a higher incidence of perineural spread of tumour than had previously been recorded. He demonstrated this was due to the tissue sampling technique used in his scheme for chemotherapy of superficial cancers. Of 1734 squamous carcinoma cases, he found forty-one with the perineurium involved, but the histology did not show keratinizing squamous cell carcinoma within the nerves, as in our case.

Pain was a marked symptom in this case, though initially its significance was not appreciated. At the time of the patient's first consultation, tenderness was noted and this is an unusual symptom in keratoacanthoma. When the patient was seen after the initial curettage of the tumour, pain in the finger, hand and forearm continued to dominate the symptoms. Paraesthesia in the finger tip was first noticed about 6 months after the appearance of the skin lesion, yet 10 months later, when the arm was amputated there was no anatomical or histological evidence of continuous spread proximally along the nerves from the site of the original lesion.

Another interesting feature of the case was the variable degree of differentiation of the tumour from one specimen to the next. The initial nodule was well differentiated squamous epithelium and, even on review, it was consistent with keratoacanthoma. The second biopsy from the finger, and later the tumour within the median nerve, was well differentiated and producing organized keratin, yet the intermediate biopsy sample from the carpal tunnel showed poorly differentiated squamous cell carcinoma.

This case appears to be the first recorded example of a squamous cell carcinoma of skin arising elsewhere than the head, neck or penis that has spread along a peripheral nerve. It is also unusual in the absence of obvious direct extension from the primary tumour. Median nerve compression at the wrist by a neural metastasis does not appear to have been reported previously; yet another condition must be added to the list of possible causes of the carpal tunnel syndrome.

ACKNOWLEDGMENTS

Our thanks are due to Mr C.W.S.F.Manning and Mr J.O.Robinson for permission to report this case, and to the Photographic Departments of the Institute of Orthopaedics and St Bartholomew's Hospital.

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Lupus erythematosus in association with finger-clubbing

RONA M.MACKIE

University Department of Dermatology, Western Infirmary, Glasgow

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SUMMARY

A case is reported in which finger-clubbing was associated with, and apparently caused by, lupus erythematosus.

Despite the frequency of involvement of the skin of the dorsal areas of the fingers in lupus erythematosus, there are no reports of associated finger-clubbing. A patient is therefore reported with this association in whom careful investigation has failed to reveal any other underlying cause for the finger-clubbing.

CASE REPORT

The patient, a female born in 1919, presented in 1969 with a 5-year history of painful swollen finger-tips, and a 2-year history of hyperkeratotic lesions on the palmar and lateral surfaces of the fingers. Her general health was good and her only other dermatological complaint was of intolerance of sunlight, which caused marked facial erythema and discomfort. There was no family history of finger-clubbing. Specific enquiry yielded no symptoms attributable to cardiac or gastro-intestinal systems, and no features in the history were indicative of thyroid disease. There was no past history of cardiac or respiratory problems, including tuberculosis.

Examination revealed moderately severe finger-clubbing affecting all digits (Fig. 1). In addition to the hyperkeratotic lesions, small areas of atrophy were observed on two fingers, and in the peri-ungual area of three digits small haemorrhagic lesions were visible. No other dermatological abnormalities were observed, and general examination was likewise negative.

The positive laboratory findings on investigation at this time were a positive antinuclear factor at a titre of 1 in 256, a haematological preparation for LE cells containing several highly suspicious cells, and a skin biopsy from the left index finger showing hyperkeratosis, basal layer liquefaction and a dermal lymphocytic infiltrate consistent with but not diagnostic of lupus erythematosus (Fig. 2). X-ray of the hands showed soft tissue swelling but no bony abnormality, and no periosteal reaction at the ends of the radius or ulna. The ESR was 9 mm in the first hour and chest X-ray, ECG, pulmonary function studies, thyroid function studies, porphyrin levels, haemoglobin, white cell count, platelet count, urea, electrolytes, liver function studies, serum calcium, phosphate, and carotene levels were all within normal limits.

In view of these findings, further histological studies were carried out on both involved and clini-

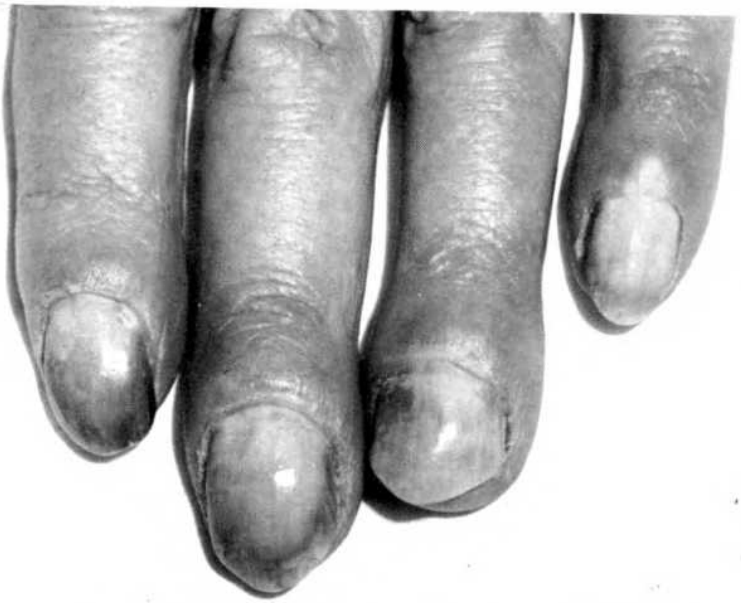

FIGURE 1. Clubbing is seen most markedly in third finger.

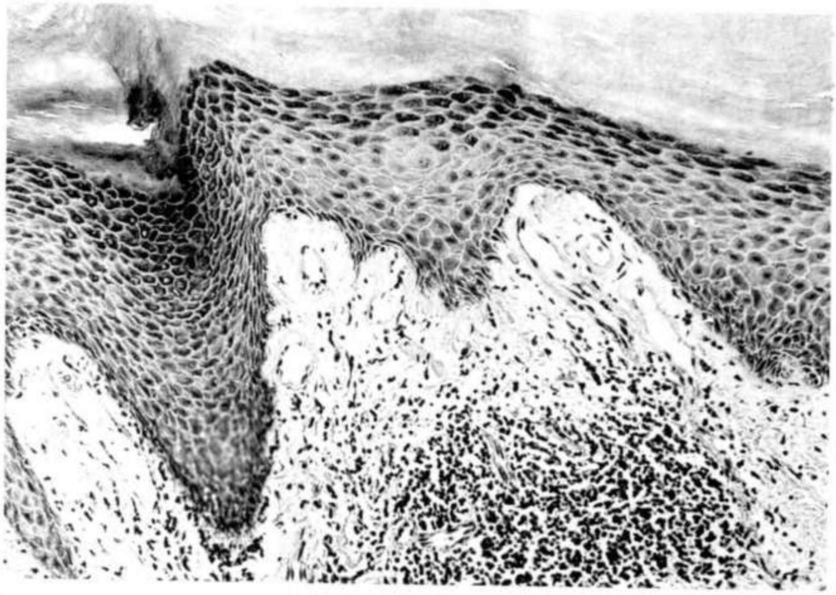

FIGURE 2. Histology of skin adjacent to nail fold, showing hyperkeratosis, liquefaction degeneration of the basal layer and lymphocytic dermal infiltrate. Dilated capillaries are also seen in the papillary dermis.

cally normal skin. Basal lamina immunofluorescence was sought, using sheep antihuman IgG conjugated with fluorescein isothiocyanate. Immunofluorescence was observed at the basal lamina region of both the involved and the uninvolved skin.

In view of the lack of evidence of multisystem involvement no therapy was instituted, but the patient was seen at regular intervals. In the summer of 1970 photosensitivity became troublesome, and after lack of adequate response to topical preparations containing mexenone and potassium para-amino benzoate, a trial of systemic chloroquine phosphate was instituted, with immediate improvement in ability to tolerate sunlight. Because of this observation antimalarial therapy was continued over the summer months of 1971 and 1972, with good effect. At present the condition of the patient's nails has not changed since her presentation, although new hyperkeratotic lesions have appeared on the surrounding skin, and pre-existing hyperkeratotic areas have progressed to atrophy. Repeat detailed laboratory investigation in January 1973 showed no evidence of abnormality of pulmonary, hepatic, gastro-intestinal or renal function. Haematological findings were likewise normal, and again on this occasion no diagnostic cells were observed in an LE cell preparation. However, the antinuclear factor was still positive, in a titre of 1 in 256.

DISCUSSION

Despite the frequency of involvement of the skin of the hands in both the systemic and the chronic discoid varieties of lupus erythematosus there appears to be no report of finger-clubbing in association with either condition. Samman (1972) discusses the erythema and telangiectasia in the peri-ungual region which are not infrequently observed in systemic lupus erythematosus. There is one report of cparonychia, proceeding to onychia, insystemic LE (McCarthy, 1930), and Heller (1927) reported one case of gross nail dystrophy with associated deformities of the terminal phalanges. By contrast the patient here described shows no dystrophy of the nail itself.

Throughout the 4-year period over which this patient has been observed, the possibility of her finger-clubbing being due to thyroid pulmonary or gastro-intestinal dysfunction, has been regularly considered, but repeated investigations have yielded negative results, and as the clubbing has now been present for 8 years, it seems unlikely that a condition such as carcinoma of bronchus or bronchiectasis would not have declared itself in this period.

The condition would appear therefore to be either 'idiopathic' or associated with lupus erythematosus.

The basic process giving rise to finger-clubbing in the pulmonary, cardiovascular and endocrine conditions in which it is observed is not established, but in many cases some degree of anoxia would appear to be at least in part responsible. From the relatively normal appearance of the nails themselves it would appear that the matrix is not involved, but the possibility of involvement of the blood vessels in the finger pulp in the LE disease process giving rise to a degree of hypoxia seems a reasonable hypothesis.

ACKNOWLEDGMENTS

I should like to thank Dr N.K. Saikia for carrying out immunofluorescence studies on this patient.

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Comment

TISSUE INTERRELATIONSHIPS IN CUTANEOUS GRANULOMA

The most appropriate introduction to this difficult subject is possibly the pithy statement of Epstein: 'Granuloma formation remains an enigma' (Epstein, 1967). The same sentiment is expressed more precisely by Spector & Lykke (1966) when they say that: 'In spite of the importance of chronic inflammation in human pathology, little is known about the natural history of inflammatory granulomata, i.e. the origin of the cells of which they are composed and the way in which the infiltration survives, develops and changes over a period of months and years'. Of course, granulomatous inflammation of the skin does not differ fundamentally from the same process in other parts of the body, but the involvement of the epidermis and the specialized nature of the dermal connective tissue may influence its course and appearance. Apart from the intrinsic interest of the subject, a prime reason for its especial appeal to dermatologists is the occurrence of granulomatous inflammation in a wide variety of quite common dermatoses.

The term granuloma is derived from the granular appearance of healing wounds. It has subsequently been extended to describe localized, persistently inflamed areas that not only contain polymorphonuclear leukocytes, lymphocytes and plasma cells but also, in varying proportions, macrophages, epithelioid cells, giant cells, fibroblasts and newly formed blood vessels. In this context it may be useful, as recently emphasized by Pinkus & Mehregan (1969) to keep in mind the old term 'productive inflammation'. This encourages a dynamic concept of the formation of cell constituents of the granuloma, though the implication that the components are produced locally is no longer valid. This 'definition' of a granuloma makes it possible to include such lesions as histiocytoma and juvenile xanthogranuloma. These lesions are, from a morphological point of view, in a sort of twilight zone between neoplasms and granulomata. Although they may bear little resemblance to inflammatory foci, and it is undoubtedly extremely difficult with individual lesions to give a firm opinion, it is probably fairest to regard them as unusually persistent and indolent granulomata. Lesions that have a disproportionate content of only one tissue component, such as the blood vessels in pyogenic granuloma, are best excluded from the granulomata.

It has been considered that granulomatous inflammation is a response to injury by a comparatively insoluble material which evokes proliferation of reticulo-endothelial cells. A particular problem with granulomatous inflammation of the skin is the wide range of agents that can evoke an apparently identical morphological response. Although a definite aetiological agent can be unearthed in many cases, there are several common granulomatous conditions the cause of which can only be surmised. Amongst these are sarcoidosis, granuloma annulare, necrobiosis lipoidica and rosacea. It should be noted that this group may be troublesome clinically, as only empirical therapy at best is available for their treatment. It is probably fair to suggest that an understanding of the sequence of events in granulomatous reactions and the role of each tissue component, are essential for designing methods of treating these disorders.

In this comment we have selected for discussion only a few aspects from the whole problem of granuloma formation. We have chosen the interactions between dermal granuloma and the overlying epidermis because this subject has not often been discussed and is of considerable interest. We will also discuss briefly the role and behaviour of blood vessels, as we believe that this is an important and neglected aspect of the granuloma problem. The third subject upon which we have decided to comment is the interrelationship of the inflammatory cells comprising granulomata, as this topic is central to the pathogenesis of granulomatous inflammation.

EPIDERMAL INVOLVEMENT

The epidermis overlying granulomatous lesions may exhibit a variety of changes which are easily recognized but difficult to explain. Both epidermal hyperplasia and epidermal atrophy may be seen above granulomata, and to make matters more confusing, both these changes may be present in different sites over the same granuloma! It is nonetheless true that certain granulomata are more likely to be accompanied by particular epidermal responses than by others, for example, the pseudo-epitheliomatous hyperplasia or the warty hyperplasia found over particular types of cutaneous tuberculosis.

Epidermal atrophy or thinning can be seen in two situations. It is evident over some dermal lesions, expanding or not; distortion of the vascular supply to the epidermis could be partially responsible for this change in epidermal architecture. It is also seen focally over some granulomata where the inflammatory process impinges directly on the epidermis and eventually erodes it. The first type is exemplified by necrobiosis lipoidica and the second by a variety of lesions, including some foreign body granulomata. Epidermal hyperplasia is of more interest to us because it has been possible to induce this change experimentally in guinea-pigs by injecting them intracutaneously with carrageenan, silica or beryllium (Schellander & Marks, 1973a). It was shown that the epidermis produced a proliferative response within 2 days of the induction of the focus of inflammation, and at the same time became considerably thickened. In the course of this hyperplasia it was also demonstrated that 'high level' intra-epidermal cells contributed appreciably to the autoradiographic labelling index after exposure to tritiated thymidine (Schellander & Marks, 1973b). It was suggested that this was due to a concomitant proliferative response of Langerhans' cells. A similar study examined the effect that dermal granulomata had on melanocytes in the overlying epidermis (Eady & Marks, 1973). A decrease in the number of DOPA positive cells relative to the number of basal epidermal cells was found in most sites, although this was not a uniform change, as in some areas there was an increased number of DOPA-positive cells.

The epidermal alterations over histiocytomata require special mention. It is usual to see acanthotic thickening over histiocytomata and this may be accompanied by a variable amount of hyperkeratosis. A seborrhoeic wart-like change is quite commonly seen as well. Less commonly, a very distinctive type of basaloid hyperplasia occurs over these dermal lesions; in at least two cases this has progressed to genuine basal cell epithelioma (Caron & Clink, 1964; Yanowitz & Goldstein, 1964). Pinkus has suggested that the basaloid hyperplasia represents the induction of new hair follicles (Pinkus, 1967), but there is little evidence to support this concept. Uncommonly, no epidermal thickening takes place and atrophy is seen instead.

Pseudo-epitheliomatous hyperplasia is a particularly exuberant epidermal reaction that quite often accompanies deep fungous infections such as North American blastomycosis. It may also be seen accompanying foreign body granulomata. At times there can be a striking resemblance to true neoplasia and this, taken with the regularity of its appearance over some lesions, suggests that there is a special stimulus for this change rather than an exaggeration of the ordinary hyperplastic response.

There is one other way in which the epidermis can become involved in granulomata of the skin. When a focus of inflammation is immediately subepidermal and is produced by irritant particulate material, the epidermis tries to eliminate this material by enveloping it in epidermal prolongations.

This process has come to be known as transepidermal elimination or 'phagohistosis'. It is seen in the extrusion of subepidermal calcium deposits resulting from various disorders, in some foreign body granulomata and in granulomata due to 'deep fungous infections'. It is also seen classically in Miescher's elastoma. It is an astonishing activity involving complicated epidermal movement and re-organization. We know nothing of the mechanisms involved, but it is possible to induce it experimentally by the deposition of particles of Indian Ink subepidermally (Schellander & Marks, 1973c).

THE ROLE AND BEHAVIOUR OF BLOOD VESSELS IN GRANULOMATA

As pointed out above, granulomata were so called because of the macroscopic resemblance to the 'granulations' seen in healing wounds. These granulations are red and have a dominant vascular component. Clearly, blood vessels proliferate alongside the other components of granulomatous inflammation, but nothing is understood of the stimulus that provokes them to do this. In fact, remarkably little is known concerning the mitotic activity of human vascular endothelium, stimulated or unstimulated. Tumour research has established that some neoplasms produce a factor or factors capable of stimulating new vessel growth (Cavallo *et al.*, 1972; Warren, Greenblatt & Kommineni, 1972); possibly a similar mechanism is responsible in granulomata. The investigation of this type of problem is fraught with difficulties, because there is no sure way of identifying vascular endothelium in histological sections. One way around this problem, which is capable of providing limited information, is to score only those cells that line the lumen of a vascular space. One of us adopted this approach during an autoradiographic and histochemical investigation into the histogenesis of the inflammatory component of rosacea (Marks, 1973). In this study, endothelial and perithelial cells were shown to be labelled autoradiographically after intracutaneous injection of tritiated thymidine. Other workers have noted that these cell types become labelled in experimental granulomata (Spector & Lykke, 1966). Tannock & Hayashi (1972) used Luxol Fast Blue to identify endothelium in regenerating callus, but this is not so easy as it sounds. Other workers have examined proliferating vascular endothelium by a combination of the use of 'semi-thin' sections 1 μm thick, embedded in epoxy resin, and autoradiography. However, even with this technique, identification of endothelium is not completely certain and some investigators believe that electron microscopic techniques are essential. One further technique that may be employed depends on the increased permeability of small inflamed blood vessels to circulating India Ink particles.

It is often suggested that the caseation necrosis seen in tuberculosis is the result, at least in part, of the avascularity of the lesion. There appears to be some evidence for this (Florey, 1970). However, vascular factors cannot entirely explain caseation necrosis. If they did, why should there be extensive necrosis in acne agminata but none in rosacea, or necrosis in some types of cutaneous tuberculosis but hardly ever in lupus vulgaris? It is perhaps relevant to note in this respect that although vascular changes are thought by many to be responsible for the necrobiotic change seen in the granuloma of necrobiosis lipoidica, no such vessel abnormalities are evident when this disorder occurs on sites away from the legs (Wilson Jones, 1971). Possibly the most important role that vessels play in granulomatous inflammation is the constant supply of new inflammatory cells.

ORIGIN AND FATE OF INFLAMMATORY CELLS

A tremendous amount of research has been lavished on the kinetics of cellular infiltrates in granulomata and in the present commentary it will be possible only to pick out some important points. In particular, we will deal with the sources of macrophages, epithelioid cells, giant cells and fibroblasts, and with interrelationships between these cell types. The role of hypersensitivity in the production of some types of granuloma will also be considered, as immune mechanisms are concerned in their production.

The weight of evidence strongly favours the view that the majority of mononuclear cells making up chronic inflammatory infiltrates come from the blood and ultimately the marrow (Spector, Walters & Willoughby, 1965; Volkman, 1966; Spector & Lykke, 1966). Experiments involving X-irradiation of the marrow of rats pointed to this tissue as being the most important source of the monocyte series of cells (Volkman & Gowans, 1965). Van Furth & Cohn (1968) showed that even in the 'steady state' most fixed tissue monocytes were derived from the proliferating monocyte population of the marrow. Rebuck *et al.* (1964) believed that the lymphocyte series was a source of many of the phagocytes in chronic inflammation. It is, of course, extremely difficult to distinguish small mononuclear phagocytes from lymphocytes. However, an ingenious experiment that involved the labelling of circulating cells autoradiographically with tritiated thymidine and with carbon particles demonstrated convincingly that haematogenous mononuclear cells and not lymphocytes were the precursors of phagocytic cells in chronically inflamed lesions (Spector *et al*, 1965). The haematogenous source of the inflammatory infiltrate runs contrary to the traditional view of the origin of these cells. It was believed that they originated from the multiplication of local 'fixed tissue cells' and this is how the term 'productive inflammation' arose. It is certainly true that when a chronically inflamed lesion is exposed to tritiated thymidine a proportion of the cells can be shown to be in DNA synthesis if the tissue is examined autoradiographically. Lachapelle (1972) has found, for example, that the infiltrate in primary irritant dermatitis has a labelling index of 0·5%, in contrast to the index of 2·5% in allergic contact dermatitis. The fact that cells in inflammatory foci are capable of division does not imply, as some have suggested, that they arise locally. It merely signifies that many of the cells that arrive at the inflamed area are not 'end' cells. It is obvious that the number of cells that become labelled after exposure to a 'pulse' of tritiated thymidine a short time before the tissue is fixed is a function of the 'turnover' of those cells. Similarly, if the cells have been labelled by exposure to the thymidine some time previously, then the rate of disappearance of labelled cells from the tissue reflects their rate of destruction (and/or division) and is again a function of 'turnover'. Spector (1971) has elaborated the idea of 'high' and 'low output granulomata on the basis of these investigations. Carrageenan is a polysaccharide material derived from seaweed. This substance produces a 'low output' granuloma, presumably because of its low toxicity for the cells that phagocytose it. 'High output' granulomata were found in reactions caused by pertussis and TAB vaccines and by paraffin oil (Ryan & Spector, 1969). In the high output granulomata only a small proportion of the macrophages contained the irritant material. This description of the kinetics of the infiltrate in granulomata is of importance when it comes to a discussion of hypersensitivity in relation to granulomata, as hypersensivity granulomata appear to be of the 'high output' type.

The epithelioid cell is so called because its large vesicular nucleus and the lack of a distinct cell periphery give it a superficial resemblance to an epithelial cell. It has been shown in experimentally induced granulomata in mice (both *in vivo* and *in vitro*) that epithelioid cells are derived from mononuclear types (Papadimitriou & Spector, 1971). Similarly, Epstein & Krasnobrod (1968) found that in beryllium and zirconium granulomata in man mononuclear cells became autoradiographically labelled when biopsies were taken a short time after injection of tritiated thymidine, but when biopsies were taken 2 weeks after injection, most of the label was situated over epithelioid cells. Although epithelioid cells possess strong hydrolytic enzyme activity and much of the subcellular apparatus of macrophages, they do not seem to have a phagocytic role (Elias & Epstein, 1968; Papadimitriou & Spector, 1972). It seems that they develop when macrophages 'become immobilized at a site of inflammation without being called upon to undertake phagocytosis, or when phagocytosis or pinocytosis leads to complete elimination of the particle within a few days' (Papadimitriou & Spector, 1971).

Although epithelioid cells may form in animals whose immune apparatus has been destroyed, it appears that when masses of these cells are organized into an 'epithelioid cell granuloma', hypersensitivity plays a dominant role in the pathogenesis of the lesion. Experimental work suggests that a true epithelioid cell granuloma (as opposed to a foreign body granuloma) develops only as a result of a specific acquired hypersensitivity. This certainly appears to be true for the zirconium granulomata (Shelley & Hurley, 1958) seen as a result of using one type of deodorant. It is also true for the granulomatous responses seen after injury by fluorescent light tubes containing beryllium compounds (Epstein, 1967). Epstein believes that it is important to distinguish this type of granulomatous hypersensitivity from foreign body granulomata. There are very few data concerning the relationship of delayed hypersensitivity to granulomatous hypersensitivity. Presumably, whether a conventional delayed hypersensivity reaction or a granulomatous reaction develops is a function of the physical state of the antigen and of its effect on macrophages once it has been phagocytosed. Epstein (1967) suggests that lymphocytes transfer a 'message' to macrophages which induces them to become epithelioid cells.

Little is known concerning giant cell formation. Ryan & Spector (1970) found that the nuclei in giant cells were capable of division, but Gillman & Wright (1966) found that the giant cells of foreign body granulomata did not divide. These giant cells seemed to form by fusion of mononuclear cells.

The repair of damage produced during 'the battle' that is granulomatous inflammation proceeds quietly and continously. The fibroblasts that synthesize the new connective tissue components may also be derived from the macrophage series. Kouri & Ancheta (1972) have shown by examining ultrastructurally the cells that adhere to implanted laminae that there are many intermediate cell types between macrophages and fibroblasts. Many factors control scar formation; amongst them may be listed the extent of the damage, the rapidity of its development and its depth within the dermis. There must be other considerations which we do not understand at present, or else why should necrobiosis lipoidica but not granuloma annulare lead to scar formation. Similarly, why should rosacea, however severe, not cause scarring although scarring is quite common in acne agminata.

We hope that in this brief incursion into a complex but important field, we have at least succeeded in summarizing some of the more salient features concerning cutaneous granuloma formation. The stress on what is not known has been intentional, as this is still an area of profound ignorance.

F. SCHELLANDER
R. MARKS

Department of Experimental Pathology
Institute of Dermatology
St John's Hospital
Leicester Square
London WC2H 7BJ

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Correspondence

ULTRASTRUCTURAL OBSERVATIONS ON UNINVOLVED SKIN IN DERMATITIS HERPETIFORMIS. 'ELASTIC' VERSUS 'RETICULAR' FIBRILS

SIR, We should like to comment on the article 'Ultrastructural observations on uninvolved skin in dermatitis herpetiformis' recently published in this Journal by Riches *et al.* (1973). The authors referred to as 'reticular', groups of fibrils located in the superficial dermis and ascending vertically to the dermo-epidermal junction. However, the term 'reticular' is usually given in histology to fibres that, when

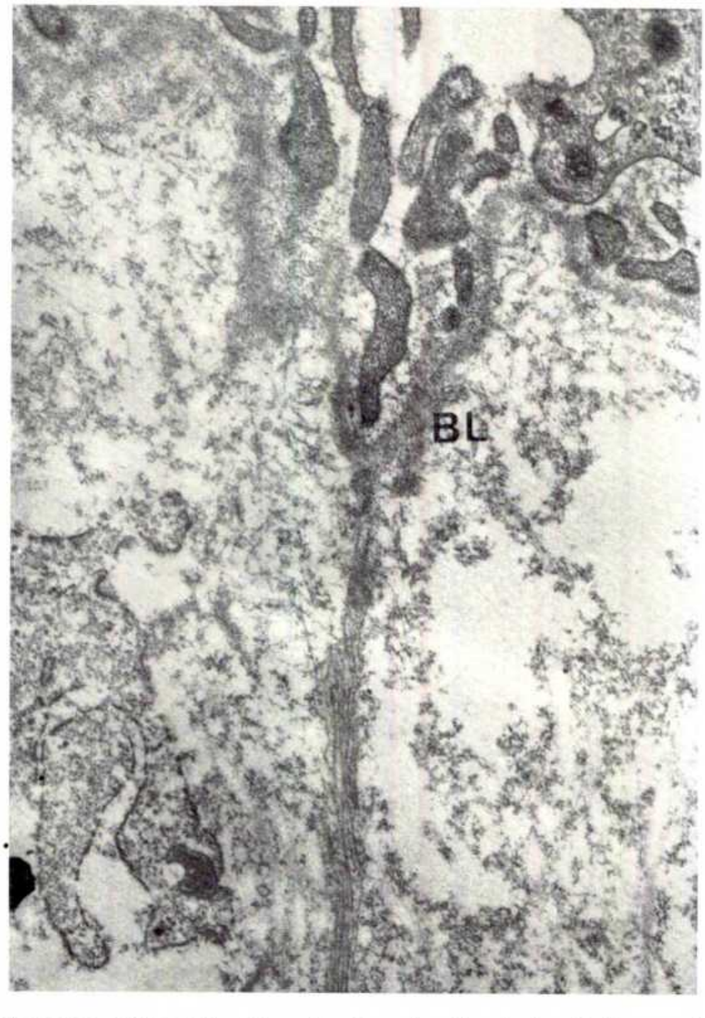

FIGURE 1. The dermo-epidermal junction, showing a bundle of microfibrils ascending vertically to the basal lamina (BL) (× 25,600).

examined in the electron microscope, reveal the periodicity of collagen, differing from it only in their arrangement and histological staining reactions.

We studied bundles of fibrils with a similar location in normal skin and in lesions from dermatitis herpetiformis, and we found that each fibril is about 10 nm in diameter and, at high magnification, shows a tubular appearance (Figs. 1 and 2).

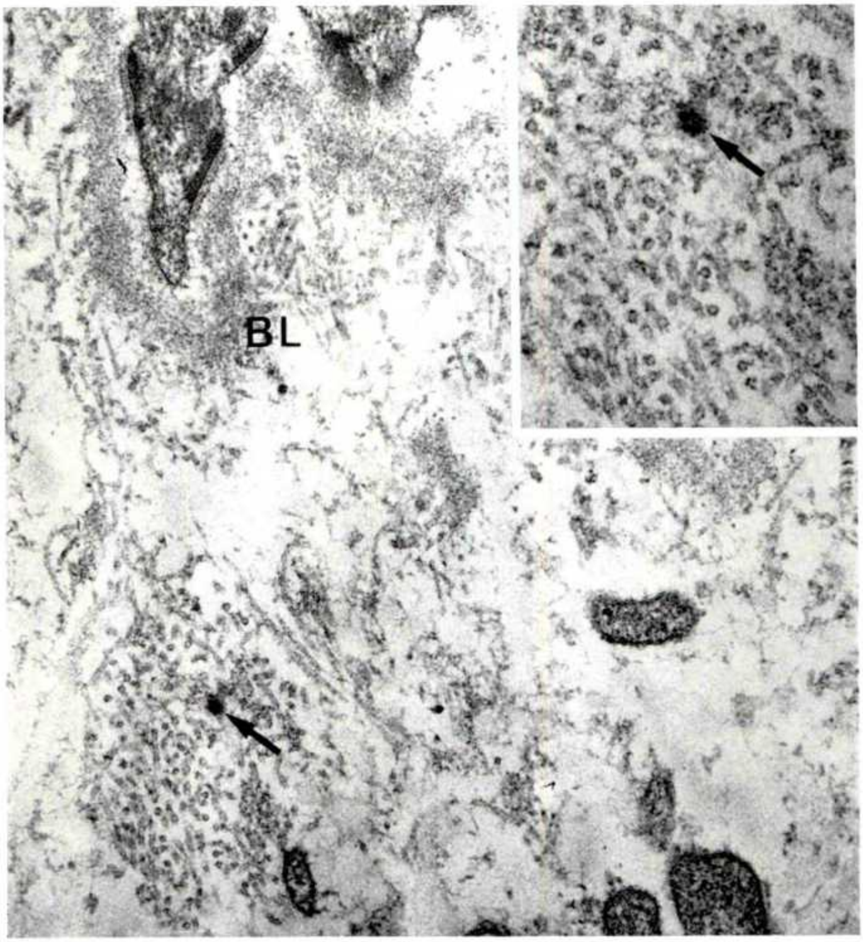

FIGURE 2. A bundle of microfibrils has been sectioned transversely. Note their tubular appearance (insert), and the small amorphous structure (arrows), which is probably elastin (× 44,800).

It is known that typical elastic fibres are formed by amorphous elastin surrounded by tubular structures of approximately the same diameter as the fibrils studied here (Greenlee, Ross & Hartman, 1966; Ross & Bornstein, 1969). For this reason, we think that the groups of fibrils to which Riches *et al.* refer are the fibro-tubular component of elastic fibres devoid of elastin.

On optical microscopy it is possible to reveal these bundles with the specific stains for elastic tissue after oxidation with peracetic acid (Hasegawa, 1960; Sampaio & Pereira, 1971; Pereira, Rodrigo & Sampaio, 1973); thus they may be classified as oxytalan fibres (Fullmer & Lillie, 1958; Carmichael & Fullmer, 1966).

It is known from ontogenetic studies that the fibrillar component of the elastic fibres appears before the amorphous one (Ross & Bornstein, 1969; Albert, 1972). In our opinion the bundles of fibrils observed in the superficial dermis may correspond to an arrest in the development of the elastic fibres (Pereira *et al.* 1973).

F.GUERRA RODRIGO
GERSON COTTA-PEREIRA

Laboratory of Cell Biology
Gulbenkian Institute of Science
Oeiras, and
Dermatologic Clinic
Faculty of Medicine
Lisbon, Portugal

The comments and findings of Rodrigo and Pereira are interesting and may be relevant to the development of the lesions in dermatitis herpetiformis. While their interpretation of the microfibrils as components of elastic fibres is possible, the evidence so far presented is not conclusive. It is fair to say that I, together with other colleagues, have rarely seen elastic fibres so close to the dermo-epidermal junction in normal skin and other pathological conditions.

D.J.RICHES

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PEMPHIGUS AND MYASTHENIA GRAVIS

SIR, The paper by Vetters *et al.* (1973) is of great interest. We can all appreciate how, in spite of one's best efforts, lists are seldom complete; it is for information only that I draw attention to a case report (Ridley, 1970) of pemphigus vulgaris with myasthenia gravis (and membranous colitis). I note too that MacKie, Saikia & Morley (1973) mention in addition a case report by Jablonska (1970).

MARJORIE RIDLEY

Whittington Hospital
London, N.19

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Book Reviews

Immunology, Immunopathology and Immunity. STEWART SELL (1972) Hagerstown, Maryland: Harper and Row. Pp. viii+277, 45 illustrations. Price £6.05

Primarily intended for the medical student, this book is a good introductory text on immunology and its role in disease. The subject is presented in three parts, the first analyzing the immune reaction with chapters on such topics as antigenicity, antibody–antigen reactions, tolerance and phagocytosis. The chapter on lymphoid tissue and the immune response is particularly good.

The second part, immunopathology, discusses the immunological mechanisms of tissue damage, using basically the Gell and Coombs classification of cytotoxic, anaphylactic, toxic complex and delayed hypersensitivity reactions. To these, however, Sell adds neutralization or inactivation of biologically active molecules, and granulomatous reactions. As examples of the former he includes antibodies to insulin and blood clotting factors, and long-acting thyroid stimulator (LATS). Granulomatous reactions, as are seen in sarcoidosis, leprosy and berylliosis, appear to occur as a response to poorly soluble antigens. However, the relation of this type of reaction to other hypersensitivity reactions requires clarification.

The final section of the book covers immunity to infection, cancer immunology and immune deficiency diseases.

The material presented is generally clear and authoritative, although its selection and treatment are somewhat uneven, particularly in the latter two sections of the book. The clinical dermatologist will find the chapter on drug allergy superficial, and Table 14–4 might have been updated by the inclusion of data on pemphigus and bullous pemphigoid. The text is accompanied by good illustrations, an adequate index, and well selected references at the end of each chapter.

Despite minor criticisms the book is recommended to the clinician who wants a concise overview of current immunology and immunopathology.

H.K.MULLER

New Periodicals

Allergologia et Immunopathologia.

This new journal, published in Madrid, is the official organ of the Spanish Society of Allergy and the Latin American Society of Allergy and Immunology. It is the successor of the Revista de Allergologia, founded in 1951, which ceased publication 6 years ago. The editors are A.Oehling of Pamplona and E.Mathov of Buenos Aires. Britain, France, Germany, Holland and the U.S.A. are represented on the Editorial Committee or provide at least one Associate Editor. Appropriately, Spain, Portugal and the countries of Latin America make the largest contribution to the team's membership.

Initially this journal will be published six times each year at an annual subscription of Ptas 1500 in Spain and the rest of Europe, or $25 in other countries. Orders should be sent to Allergologia et Immunopathologia, Apartado 1038, Madrid.

Papers are published either in English or in Spanish with a detailed summary in the other language. Too often other-language summaries are at best a translation of the author's summary in the language of the paper. Since such summaries are intended specifically to help those who cannot read the paper itself they should be long and informative. It is encouraging to find that the editors of this journal appreciate the importance of this neglected aid to international communication. The summaries are indeed detailed. It should be easy with the help of the English speaking associate editors to ensure that in future they are written in clear grammatical idiomatic English.

This journal, which covers both the laboratory and clinical aspects of allergy and immunology, has already published papers of interest and importance. It is lavishly produced and is attractive to read and to handle.

British Journal of Dermatology (1973) 89, 549.

Quarterly Review

DRUG REACTIONS XXIII

Drug Induced Alopecia

Disturbances of hair growth by chemical agents are becoming increasingly common in medical practice.

Certain drugs, e.g. antimitotic agents, interfere with hair growth in the anagen or proliferative phase, and alterations in the normal structure of the anagen root can be seen microscopically (Van Scott, 1958). The mode of action in the case of other drugs is uncertain, and a logical classification of drugs causing alopecia is therefore difficult.

COMPOUNDS INTERFERING WITH THE DEVELOPMENT OF HORNY STRUCTURES

Thallium

Thallium sulphate was used in the late nineteenth century for the treatment of many conditions, including tuberculous night sweats, syphilis and gonorrhoea, when its action on hair was first noticed. Subsequently, because of its depilatory effect, it was used in the treatment of ringworm of the scalp. Poisoning was frequent because of the narrow margin between the therapeutic and toxic dose, and its use fell into disrepute.

Thallium is not contained in any preparations that the public can buy in Britain, but is still available as a rodent poison in the U.S.A. and other countries, where accidental poisoning may still occur from contamination of grain stores (Reed *et al.*, 1963), or of other foods (Steinberg, 1961).

Loss of hair begins approximately 10 days after ingestion of thallium sulphate, and complete depilation occurs at 1 month. Axillary and facial hair and the inner third of the eyebrows are often spared (Reed *et al.*, 1963).

The mechanism of the depilatory effect is unknown, but it seems that thallium inhibits the utilization of cystine, or its incorporation into the keratin molecule and, therefore, may be regarded as an antikeratinizing agent. In rats the toxic effect can be prevented by administration of cystine (Gross, Runne & Wilson, 1948; Thyresson, 1952; Schwartzman & Kirschbaum, 1962).

The clinical manifestations in man also are compatible with the hypothesis that thallium interferes with keratin synthesis. Early hair loss, intrafollicular thinning, accumulation of air bubbles in the hair shaft followed by rarefaction or breakage of hair, indicate that the metal directly affects growing hairs (Flesch, 1963), and are characteristic signs of thallium poisoning.

The prominent histological features are dyskeratotic and necrolytic changes in the skin and hair follicles, marked parakeratosis, spongiform abscess formation and induction of telogen follicles (Schwartzman & Kirschbaum, 1962).

Vitamin A.

Vitamin A intoxication may occur from dietary reasons, e.g. excessive administration of fish-liver oils to children, or from therapeutic administration, as in the treatment of pityriasis rubra pilaris and Darier's disease. Alopecia is one of the commonest effects of hypervitaminosis A. It affects the scalp, pubic, axillary and vellus hair in a diffuse manner.

The cause of hair loss is unknown, but Flesch (1963) suggested that as the hair loss appeared late, it might be caused by a gradual interference with hair growth and not by an abrupt biochemical block as in thallium poisoning.

Antimitotic agents

Most of the drugs used in cancer chemotherapy affect the growth and metabolism not only of malignant cells, but also of certain normal cells with a high metabolic and mitotic activity, as in the matrix of growing human scalp hairs.

The main groups of antimitotic agents consist of alkylating agents (e.g. cyclophosphamide), antimetabolites (e.g. methotrexate), alkaloids (e.g. vincristine), and miscellaneous (e.g. colchicine and hydroxyurea).

Damage to the hair roots in patients treated for cancer is generally not recognized unless alopecia develops. Some patients do not become aware of alopecia until after several weeks of treatment (Falkson & Schultz, 1964). The increase in hair fall has probably begun after the usual interval of 7–14 days from the beginning of treatment, but 6–8 weeks of increased loss is necessary before the 25% reduction necessary to produce clinically evident alopecia is achieved.

No matter how the individual agent acts, its basic effect is on anagen hairs and not on hairs in the telogen phase. Small doses of drugs or chemical injuries of short duration decrease the size of the hair bulb, the keratogenous zone, or both, leading to constriction in the hair shaft. The constriction moves distally as the hair grows. Even where complete atrophy of the hair bulb occurs it is reversible if the drug is stopped. Clinically evident hair loss resulting from these drugs may occur by two different mechanisms. Hairs with atrophied roots are lost readily, either by falling out spontaneously or after casual procedures such as combing the hair.

Hairs with marked constriction of the shaft, on the other hand, break off easily at the point of constriction, leading to the characteristic anagen alopecia. The root, however, remains in the scalp, since it has already recovered from the insult of the drug and has produced the hair shaft containing the constriction.

Crounse & Van Scott (1960) found a close correlation between hair root damage and haematological signs of drug toxicity.

Certain points of interest arise with individual antimitotic agents. Lacher (1969) found partial alopecia but no complete baldness in patients receiving vinblastine. Alopecia was completely reversible on stopping the drug or on reducing the dose. The use of a head cuff to occlude scalp blood supply during, and for 5 min after intravenous injection of cyclophosphamide, in an attempt to minimize hair fall, has met with mixed success (Hennessy, 1966; Simister, 1966; Binns & Southall, 1970).

Anticoagulants

All the anticoagulants are capable of inducing alopecia (Flesch, 1963; Rook, 1965). These include the coumarins, indandiones, heparin and heparinoids. Apart from therapeutic exposure, poisoning may occur accidentally from warfarin used as a rodent poison.

The reported incidence of alopecia varies from 19% (Hirschboeck, Madison & Pisciotta, 1954) to 70% (Field *et al.*, 1961). The response appears to be related to the highest dose given rather than to the duration of treatment (Tudhope, Cohen & Meikle, 1958; Hirschboeck *et al.*, 1954). With normal therapeutic doses, most patients show increased hair fall without obvious alopecia and higher doses are needed to produce marked alopecia.

The scalp hair is mainly affected, although eyebrows, axillary and pubic regions may also be involved. The alopecia is basically of the telogen effluvium type, and recovers on cessation of therapy. Normal club hairs are shed diffusely 2–3 months after an adequate dose of the drug (Rook, 1965). The mechanism is unknown but the growing hair appears to be forced into the telogen phase (Kligman, 1961).

It has also been suggested that the antimitotic activity of heparin and related compounds (Field *et al.*, 1961) might play a part in the production of alopecia.

Hypocholesterolaemic drugs

The anticholesterolaemic agent, triparanol, may produce a syndrome comprising ichthyosis, loss of scalp and body hair and loss of hair colour (Winkelmann *et al.*, 1963). This drug has, in fact, been withdrawn from the market because of its association with the formation of irreversible cataracts. It is thought that triparanol interferes with the keratinization as a result of disturbance in cholesterol synthesis.

Clofibrate, an anticholesterolaemic drug which is used extensively, has only occasionally been reported to cause hair loss (De Gennes *et al.*, 1965).

MISCELLANEOUS DRUGS

Antithyroid drugs

These drugs include iodine, thiouracil and carbimazole. Hair loss has been reported following the ingestion of iodine in the form of inorganic iodides in cough mixtures (Chapman & Main, 1967), and also following the induction of hypothyroidism with iodine.

Loss of hair may occur in hypothyroidism resulting from over treatment with any antithyroid drugs, where it is basically due to hypothyroidism, but may occur when the patient is euthyroid. Lundbaek (1946) described four patients who developed hair loss, without signs of hypothyroidism, while receiving methylthiouracil.

Carbimazole produced a diffuse loss of scalp hair in five patients (Papadopoulos & Harden, 1966). When treatment was discontinued all patients improved. When two of the patients were given subsequent courses of carbimazole, hair loss occurred again, and in one patient improvement was noted when the dose was reduced. Substitution with potassium perchlorate or methylthiouracil resulted in improvement in some patients. All five patients were euthyroid and receiving thyroxine in replacement doses.

Oral contraceptives

Diffuse loss of hair has been associated with the use of the contraceptive pill, but incriminating evidence is conflicting. Cormia (1967) described five women, two of whom developed alopecia while receiving the pill and three after stopping the drug. Dawber & Connor (1971) described a patient who developed hair loss 2 months after stopping an oral contraceptive and again after a subsequent pregnancy. Griffiths (1973), in a study of women with idiopathic thinning of the hair, found no significant statistical difference in the number of pill takers and non-pill takers. He did, however, confirm that some women notice diffuse loss of scalp hair 2 or 3 months after they stop taking oral contraceptives. The mechanism of hair loss is unknown, but has been attributed to the state of pseudopregnancy induced by the oral contraceptive. It has thus been compared with the telogen effluvium-type of post-partum hair loss (Cormia, 1967; Dawber & Connor, 1971). However, recent studies of the effect of oral contraceptives on the hair cycle have not shown changes similar to those in pregnancy (Zaun & Gerber, 1969).

Trimethadione

Holowach & Sanden (1960) observed diffuse scalp hair loss in two patients receiving trimethadione for epilepsy. Withdrawal of the drug was followed by prompt regrowth in both patients. Pathogenesis was not discussed.

Allopurinol

Auerbach & Orentrich (1968) described a 52-year-old woman who developed hair loss and ichthyosis 6 weeks after a short course of allopurinol. They noted dystrophic telogen hair roots, and suggested that, as allopurinol was a xanthine-oxidase inhibitor (an enzyme essential for cellular metabolism), its action could be regarded as cytotoxic.

Amphetamines

Diffuse loss of scalp hair has been reported in women taking amphetamines for weight reduction (Alexander, 1965; Eckert *et al.*, 1967). The mechanism has not been explained.

Salicylates

During an investigation of coproporphyrin excretion in patients with idiopathic hair loss, Rawnsley & Shelley (1968) found unexpected blue fluorescence in the urine, which was found to have the characteristics of *o*-hydroxyhippuric acid, a urinary metabolite of salicylate. They suggested that patients with idiopathic hair loss consume more aspirin than the general population, and postulated that the drug may cause iron deficiency due to blood loss, or even may have a direct effect on hair.

Gentamicin

Yoshioka & Matsuda (1970) attributed scalp and eyebrow hair loss to gentamicin in a patient who developed other toxic manifestations simultaneously. The hair began to regrow after discontinuing the antibiotic.

Laevodopa

Laevodopa is used in the treatment of Parkinson's disease. Marshall & Williams (1971) described diffuse alopecia developing in two women receiving this drug. No mechanism was postulated.

The Middlesex Hospital,
London, W.1.
ASHLEY LEVANTINE

The Enfield Group of Hospitals
JOHN ALMEYDA

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British Journal of Dermatology (1973) **89**, 554.

News and Notices

Postgraduate Medical School, University of Cambridge

The annual introductory Course on 'The Biology of Skin' will be held in Churchill College from 10 to 14 December 1973. Applications should be sent to J.W. Woodcock, M.A., Secretary, Cambridge University Medical School, Hills Road, Cambridge.

University of London—The Institute of Dermatology
Diploma in Dermatology Examination 1973

Successful candidates were as follows: Drs Munir Gharib Al-Saddawi, Heng Leong Chan*, Hamza Juma Chum, Ralph John Day, Alan Gordin Jarrams*, Ali Mohamed Hussain Kampoori, Samir El Kinani, Marcia Wynne Llewellyn, Gloria Ramdai Maharaj, Ely Sarreal Medina, Rohani Munthe, Parvin Shahrad, Osman Mohamed Ahmed Taha, Mowfak Hashim Mahmoud Yahya, Zacharias Zacharoudes.

* Awarded a mark of distinction.

2nd International Barcelona Seminar on Advances in Dermatology

The second International Barcelona Seminar on Advances in Dermatology will be held at the Hospital de San Pablo on 4, 5 and 6 March 1974. Speakers will include T.B. Fitzpatrick, C.M. Lapière, J.M. Mascaró, H.O. Perry, J. Piñol Aguadé and Klaus Wolf. Enquiries and applications should be addressed to the Department of Public Relations, Hospital de San Pablo, Autonomous University, Avda. San Antonio M^a Claret, 167, Barcelona-13, Spain.

European Society for Dermatological Research

The ESDR meeting in 1974 will be held in Amsterdam on 23 and 24 April. Attendance at the scientific sessions will be open to all members, contributors introduced by members, and guests. Non-members who wish to present a paper are requested to send an abstract to the Secretary. They will be invited to attend the meeting as contributors or as guests. Forms for abstracts are available from the Secretary, Mons. Prunieras, Fondation Ad de Rothschild, 29 rue Manin 75019, Paris, and must be received by 15 December. Applications for membership should also be addressed to him.

British Association of Dermatologists Summer Meeting and the Anglo-French Reunion 1974

The Annual Meeting of the British Association of Dermatologists will take place in London on the 3, 4, 5 and 6 July 1974 under the Presidency of Dr Hugh Wallace. The Meeting will be combined with an Anglo-French Reunion, the Anglo-French day being the 5 July. Papers are invited and should be sent by the 1 March 1974 in the form of a summary in triplicate of approximately 200 words, to the Honorary Secretary, Dr J.S. Pegum, The London Hospital, Whitechapel Road, London E1 1BB. The *British Journal of Dermatology* hopes to publish in a Supplement the summaries of those papers which are accepted. These should therefore be detailed, precise and explicit and avoid such phrases as 'the results will be discussed'. Any references should be set out as required by the *British Journal of Dermatology*.

APPOINTMENTS

Southend-on-Sea and Chelmsford Groups of Hospitals.

Dr Angus Macmillan has been appointed Consultant Dermatologist to the Southend-on-Sea and Chelmsford Hospital Groups.

After preclinical and clinical training at Oxford he qualified M.A., B.M., B.Ch. in 1958, B.Sc. (Oxford) in 1960 and M.R.C.P. (London) in 1965. General medical training at Oxford was interrupted by 2 years of Operational Research during National Service in the Army and followed by 3 years of clinical research. In 1968 he became Registrar in Dermatology at the Royal Free Hospital for 2 years, then moved to Addenbrooke's Hospital, Cambridge, as Senior Registrar. Most of his publications are in the fields of human physiology, clinical dermatology and skin histopathology. His present interests are in clinical dermatology and ageing skin.

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Edited by J. M. Blakeway

Vol. 24 No. 11 October 1973

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N. J. Van Abbé. Eye irritation: studies relating to responses in man and laboratory animals.

J. K. Prall. Instrumental evaluation of the effects of cosmetic products on skin surfaces with particular reference to smoothness.

J. A. Kitchener. Surface forces in the deposition of small particles.

B. N. Hemsworth. Biological changes due to topical application of skin lightening compounds.

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Dermatologica

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Hůla, M. (Plzeň): Clear Cell Melanoblastoma

Gloor, M.; Oschmann, H.; Schmidt, E. und *Friederich, H.C.* (Marburg/Lahn): Beitrag zur quantitativen Bestimmung der Hautoberflächenlipide in der Praxis. 2. Mitteilung (A Practical Quantitative Estimation of Skin Surface Lipids. 2nd Communication

Collins, J.-P.; Grappel, Sarah F. and *Blank, F.* (Philadelphia, Pa.): Role of Keratinases in Dermatophytosis. II. Fluorescent Antibody Studies with Keratinase II of *Trichophyton mentagrophytes*

Ebner, H. und *Kraft, D.* (Wien): Fibrinnachweis mit einem Enzym-Antiserum-Konjugat beim Lichen ruber planus.
(Demonstration of Fibrin in Lichen ruber planus by Means of an Enzyme Antiserum Conjugate)

Sumrall, A.J. and *Johnson, W.C.* (Philadelphia, Pa.): The Origin of Dermal Fibrocytes in Wound Repair

Feuerman, E.J. and *Potruch-Eisenkraft, S.* (Petah Tikva): Kaposi's Sarcoma. A Follow-up of 38 Patients

Tagami, H. and *Ogino, A.* (Kyoto/Philadelphia, Pa.): Kerosine Dermatitis. Factors Affecting Skin Irritability to Kerosine

Short Communication · Kurze Mitteilung · Brève Communication

Haustein, U.-F. (Berlin): Ungewöhnliche Kerneinschlüsse in Lymphozyten und Basalzellen der Haut bei Lupus erythematodes
(Unusual Nuclear Inclusions in Lymphocytes and Basal Cells in the Skin in Lupus erythematosus)

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Textbook of Dermatology

Edited by
Arthur Rook M.D. F.R.C.P., D.S. Wilkinson M.D. F.R.C.P. and
F.J.G. Ebling D.SC. PH.D.

The generous reception accorded internationally to the first edition of this book has imposed the obligation on the editors to prepare a second edition considerably sooner than had been expected. The general plan of the book remains unchanged, but in some fields of dermatology very important advances have taken place in the last five years and in consequence most chapters have been entirely rewritten. It has not always been possible to omit old material to give place to new and despite efforts to restrain its growth this editon is therefore considerably larger than the first. Twelve chapters have been added to the book and several new authors have joined the team of contributors.

List of Contributors

Harvey Baker M.D.(Leeds) F.R.C.P.(Lond.)
John Martin Beare M.D.(Belfast) F.R.C.P. (Lond.)
Robert John Cairns M.B. B.S.(Lond.) F.R.C.P. (Lond.)
Robert Harold Champion M.A. M.B. B.CHIR. (Cantab.) M.R.C.P.(Lond.)
Francis John Govier Ebling D.SC. PH.D. (Bristol)
Sigfrid Fregert M.D.(Lond)
James Clark Gentles B.SC. PH.D.(Glasgow)
Roger Richard Martin Harman M.B. B.S. (Lond.) F.R.C.P.(Lond.)
Niels Hjorth M.D.(Copenhagen)
William Henry Jopling F.R.C.P.(Edin.) F.R.C.P.(Lond.) D.T.M.&H.(Eng.)
Francisco Kerdel-Vagas M.D.(Caracas) M.SC. (N.Y.)
Gunnar Lumbolt M.D.(Copenhagen)
Donald William Ross Mackenzie B.SC. PH.D.
Jack Nagington M.D.(Manchester)
William Everett Parish M.A. PH.D.(Cantab.) B.V.SC.(Liv.) M.R.C.V.S. M.R.C.PATH.
Jens Jørgen Pindborg D.D.S. DR.ODONT.
Stephen Owen Bellamy Roberts M.A. M.B. B.CHIR.(Cantab. M.R.C.P.(Lond.)
Arthur James Rook M.D.(Cantab.) F.R.C.P.(Lond.)
Neville Robinson Rowell M.D.(Newcastle) F.R.C.P.(Lond.) D.C.H.
Terence John Ryan M.A. B.M. B.CH.(Oxon.) M.R.C.P.(Lond.)
Peter Derrick Samman M.A. M.D.(Cantab.) F.R.C.P.(Lond.)
Kenneth Villiers Sanderson M.B. B.S.(Adel.) F.R.C.P.(Lond.) F.R.A.C.P.
Ian Bruce Sneddon M.B. CH.B.(Sheffield) F.R.C.P.(Lond.)
Darrell Sheldon Wilkinson M.D.(Lond.) F.R.C.P.(Lond.)
Edward Wilson Jones M.B. B.CHIR.(Cantab.) F.R.C.P.(Lond.)
Leonard Beard M.A.(Cantab.) A.I.I.P. F.R.P.S.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	percent	%
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hour(s)	h	milliequivalent	mmol		

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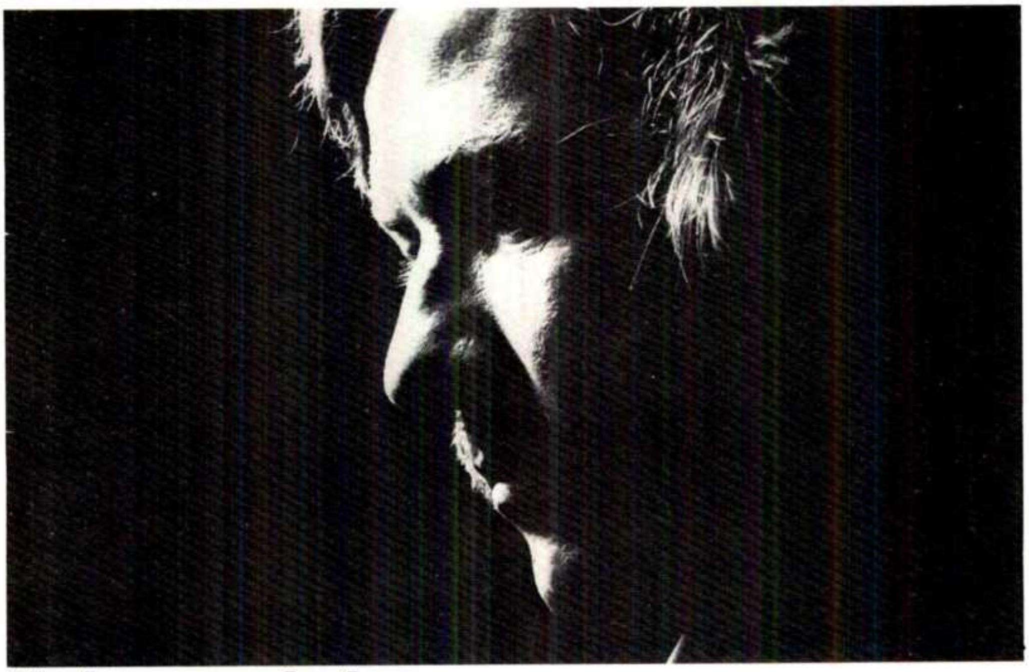
John Armstrong suffers from bouts of deep depression



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1. Brit. Med. J. (1967) 4: 535
2. CUTIS (Yorke Medical Group) (1972), 9:77

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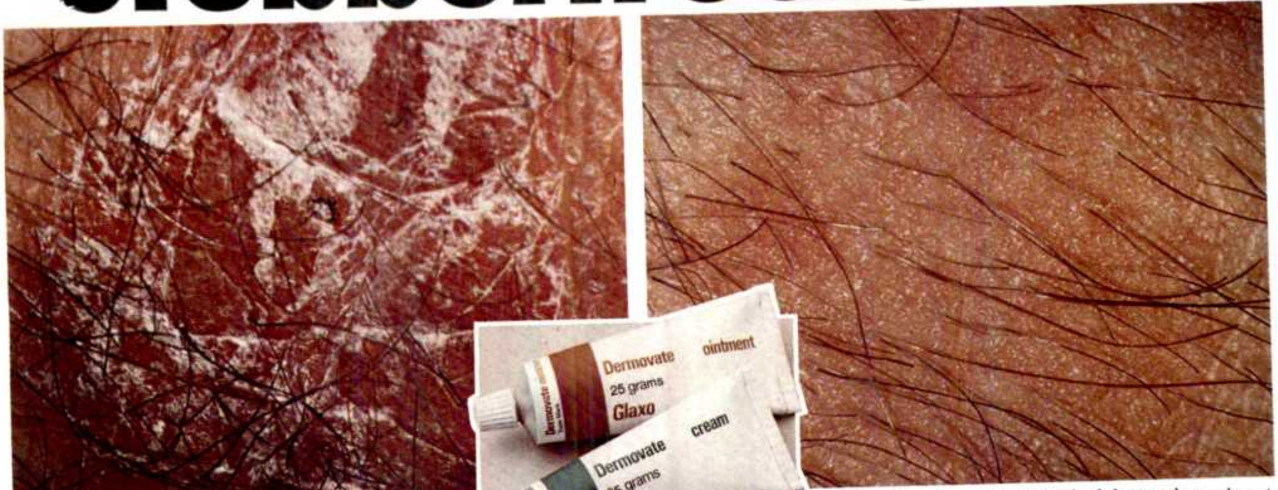

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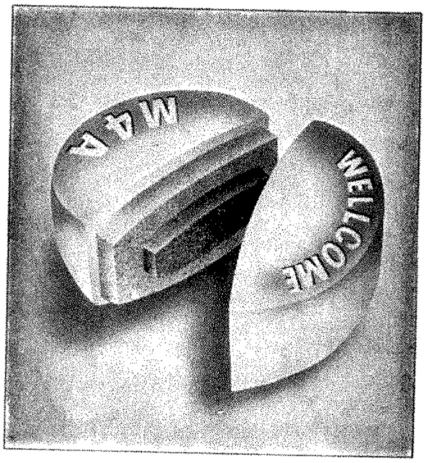

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Juvenal, Satires VII, 51.

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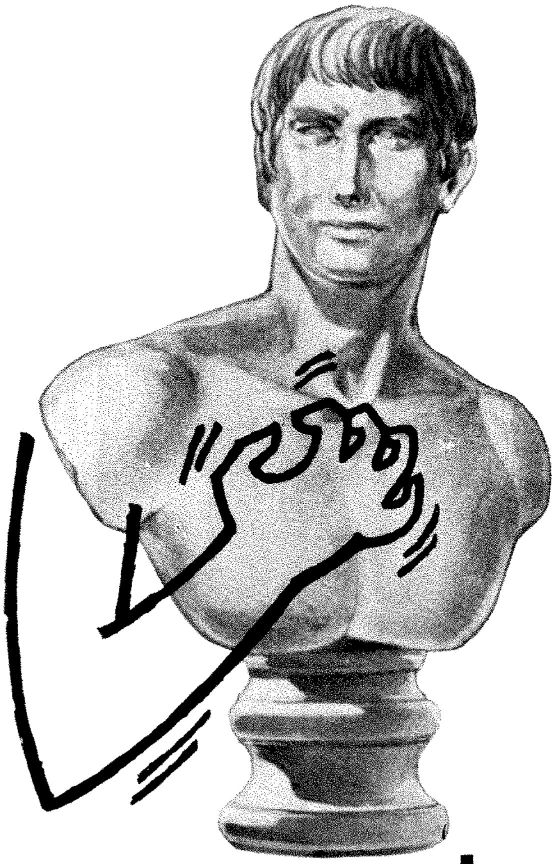

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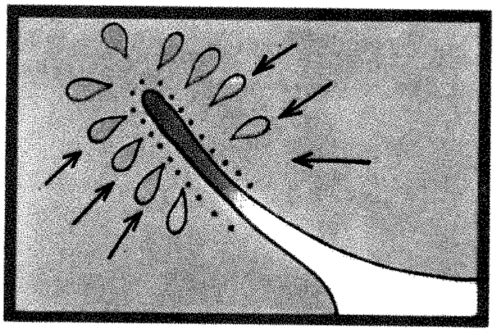

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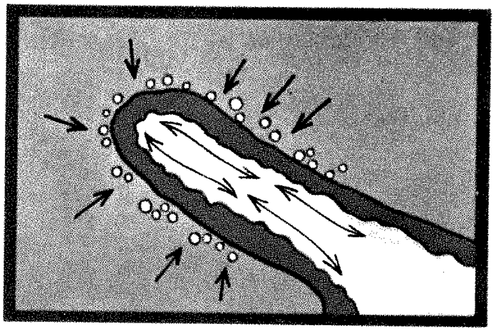

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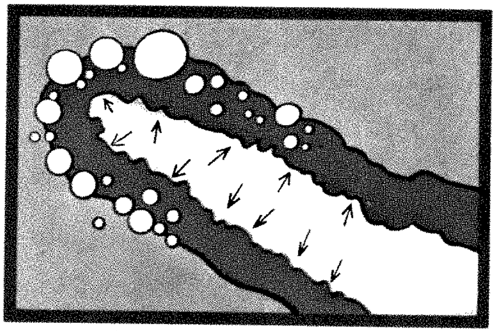

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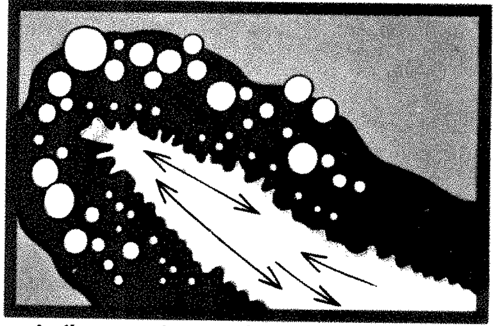

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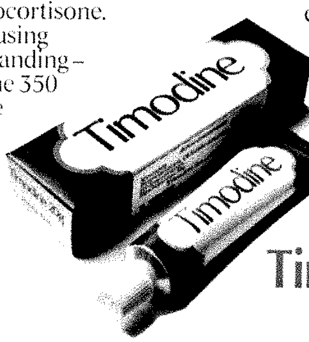

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British Journal of Dermatology (1973) **89**, 555.

Clinical and Laboratory Investigation

Anticomplementary sera in cutaneous vasculitis

J.J.CREAM*

Department of Dermatology, Middlesex Hospital, London, W.1

Accepted for publication 30 April 1973

SUMMARY

Eleven of thirty-two patients with cutaneous vasculitis had anticomplementary sera. Five of the eleven anticomplementary sera contained immune complexes in the form of mixed cryoglobulins. The anticomplementary activity in the sera of these patients suggests that the vasculitis may be due to circulating immune complexes.

Urticaria, erythema, purpura and necrosis may result from skin blood-vessel damage. From animal models it is evident that vasculitis can be due to deposition of circulating immune complexes (Dixon *et al.*, 1958), and in man the possible constituents of such complexes—antigen, antibody and complement— have been detected by immunofluorescence microscopy in the lesions (Parish, 1971). However, in purpuric lesions where the vessels are so badly damaged that red cells are able to escape, the presence of immunoglobulins or complement is of doubtful significance.

Several techniques are now available for the detection of immune complexes in serum. They include analytical ultracentrifugation (Capra, Winchester & Kunkel, 1971), electron microscopy of the sediment obtained after ultracentrifugation (Almeida & Waterson, 1969), detection of complement in the macromolecular fractions of serum that would be expected to contain complexes (Soothill & Hendrickse, 1967), and precipitation of complexes by the complement component C1q (Agnello *et al.*, 1971), or by monoclonal antiglobulin antibodies (Winchester, Agnello & Kunkel, 1970). Mixed or immune complex cryoglobulins—that is, cryoglobulins which contain two different immunoglobulins, one being an antibody against the other—also indicate that immune complexes may be present in the serum (Barnett *et al.*, 1970). These techniques have two disadvantages, in that no one test will detect all types of complexes and some require elaborate equipment.

Antibody combined with antigen is in many instances capable of fixing complement. Sera that fix added complement, without the further addition of antigen or antibody, are described as being anticomplementary. Such sera have been noted in acute post-streptococcal nephritis (Pickering, Gewurz & Good, 1968) and systemic lupus erythematosus (Castenedo & Williams, 1967); both classical examples of circulating immune complex disease. Anticomplementary activity was sought in sera from patients with cutaneous vasculitis and the results are reported here.

PATIENTS AND METHODS

Thirty-two patients with cutaneous vasculitis were studied. All had normal platelet counts. Twenty-

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* Present address: Mayday Hospital, Thornton Heath, Surrey CR4 7YE.

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one had crops of purpura of the type seen in Schönlein–Henoch purpura (Cream, Gumpel & Peachey, 1970). Two of these patients had additional features, which in one enabled the diagnosis of systemic polyarteritis nodosa to be made, whilst the other had plaques typical of erythema elevatum diutinum (EED). Two other patients who did not have crops of purpura, one with EED and the other with Degos' malignant atrophic papulosis, are included. Clinical details of the patients have been presented elsewhere (Cream, 1972a). Warm-separated sera were obtained from the patients and controls, as previously described (Cream, 1972b), and were stored at −20°C for at least 6 months before being tested.

The complement fixation test was carried out essentially as described by Roitt & Doniach (1966). Guinea-pig serum (Wellcome complement preserved guinea-pig serum) was used as a source of complement and the minimum haemolytic dose (MHD), as defined by Roitt & Doniach (1966), was determined by a preliminary titration.

Doubling dilutions of serum in buffer (prepared from complement fixation test diluent tablets, Oxoid Ltd) were made in Takatsy trays (U-shaped wells) using a microtitration kit. Three MHDs of complement were added to each well and the plates were incubated for 1 h at 37°C. Then 0·025 ml of a 3% suspension of sensitized sheep red cells was added to each well and after mixing, the plates were incubated for a further 1 h at 37°C. Controls in each batch consisted of (a) sensitized red cells in buffer without serum or complement, and (b) sensitized red cells with three MHDs of complement but no serum.

Anticomplementary activity of the serum was indicated by the presence of intact red cells. The titre was taken as the highest dilution of serum in which the button of red cells was not diminished in size.

The heat lability of the anticomplementary activity was determined by repeating the test after the serum had been heated at 56°C for 30 min.

Serum cryoglobulins were estimated as previously described (Cream, 1972b) and their components were identified by immuno-electrophoresis and double diffusion in agar.

RESULTS

Sera from twenty-two normal subjects and from thirty-two patients, who had skin diseases not thought to be due to circulating immune complexes, were not anti-complementary. Sera from thirty-two patients with vasculitis were available and eleven of these sera showed anticomplementary activity (Table 1). In all the activity was inhibited or reduced by heat. Five of the anticomplementary sera were from patients with mixed cryoglobulinaemia, but sera from nine other patients with mixed cryoglobulins were not anticomplementary. The principal components of these cryoglobulins were IgG–IgM in three sera, IgG–IgA in five sera, and IgG–IgA–IgM in one. C3 (β_1C/β_1A globulin) was detectable in three of these cryoglobulins. None of the sera was infected as shown by aerobic and anaerobic cultures.

DISCUSSION

Anticomplementary activity in the serum was demonstrated in eleven of thirty-two vasculitis patients. Such activity may be due to the presence of (a) complement-fixing material, or (b) inhibitors. Most, but not all, antigen–antibody complexes are capable of fixing complement. In addition, aggregated globulin can fix complement apparently in the same manner as immune complexes (Ishizaka, Ishizaka & Borsos, 1961), and aggregation *in vitro* may account for the anticomplementary activity that can appear in sera after storage (Polley & Mollison, 1961). Non-immune aggregation of IgG *in vivo* is a possibility and has been described in one patient with dermatomyositis (Heimer, Martinez & Abruzzo,

TABLE 1. Details of anticomplementary sera

Patient	Serum cryoglobulin* (μg/ml)	Cryoglobulin components	Anticomplementary titre of serum	
			Unheated	Heated
Ma.	770	IgG–IgM (IgA)†	1024	0
Wol.	340	IgG–IgM (C3, IgA)†	4	0
Barr.	1090	IgG–IgM (IgA)†	2	0
Pot.	305	IgG–IgM (IgA)†	32	0
Ker.	160	IgG–IgM (IgA)†	128	4
Har.	26	—	128	0
Kni.	30	—	16,384	0
Fog.‡	20	—	32	0
Byr.	14	—	512	0
Mar.§	24	—	16,384	4
Hut.	51	—	65,536	2

* Normal $<$80 μg/ml, † trace detectable by double diffusion, ‡ polyarteritis nodosa, § erythema elevatum diutinum.

1970), but appears to be very rare. A number of complement inhibitors have been found in serum. The heat labile ones include C1 esterase inhibitor and C6 inhibitor.

From the present study it is not possible to be certain which of these mechanisms is responsible for the anticomplementary activity. Aging of serum would appear to be an unlikely explanation, since none of the sera from the control groups was anticomplementary. Loss of anticomplementary activity on heating suggests that a thermolabile complement inhibitor may be involved, but isolated mixed cryoglobulins show thermolabile complement fixation (Balács & Fröhlich, 1966), and so the presence of immune complex cryoglobulins is sufficient to account for the thermolabile anticomplementary effect.

Fourteen of the sera from the vasculitis patients were known to contain immune complexes in the form of mixed cryoglobulins, yet only five of these sera were anticomplementary. There are several possible explanations. Thus, not all antigen–antibody combinations are capable of fixing complement. IgM antibodies, including rheumatoid factors, are in general good at initiating complement fixation (Schmid & Rocha, 1968). IgG antibodies vary in their ability to fix complement according to the heavy chain sub-class—IgG_1 and IgG_3 being good complement fixing antibodies, whereas IgG_2, IgG_4 and IgA fix complement poorly (Müller-Eberhard, 1968).

Complement fixation is further influenced by the antigen, in that complexes containing certain antigens—for example Rhesus antigen or thyroglobulin reacted with IgG—fail to bind complement (Lachmann, 1968). This is because in the case of IgG the antibody molecules must form a dimer before complement can be fixed, and where the binding sites on the antigen are widely separated the attached IgG molecules will be insufficiently apposed to allow this formation (Borsos & Rapp, 1965).

Other variables that may affect complement fixation are the antigen–antibody ratio (Goldsworthy, 1928), the size of the complexes and the presence of rheumatoid factor (Winchester *et al.*, 1970). Mixed cryoglobulins redissolve at 37°C. Capra, Winchester & Kunkel (1969) have suggested that the antiglobulin component of a mixed cryoglobulin is a 'cold reactive' antibody, and so the failure of complement fixation by a serum that contains an immune complex cryoglobulin may be due to dissociation of the complex at 37°C. Such sera might be expected to fix complement at lower temperatures.

However, complement fixation by IgG may be enhanced at 4°C compared with that at 37°C (Sandberg & Stoller, 1966; Cunniff & Stoller, 1968), so that increased complement fixation at 4°C by mixed cryoglobulinaemic sera need not necessarily indicate the recombination of antigen and antibody.

Complement fixation *in vivo* could result in saturation of the complement binding sites on the complex with diminished or no complement fixation *in vitro*. Three of the mixed cryoglobulins from sera giving negative results did contain human β_1C/β_1A, as shown by double diffusion.

Failure to detect anticomplementary activity could also be due to insensitivity of the test, since a large excess of complement was added and this would be additional to any complement that remained in the serum after storage.

Some of the sera had considerable complement binding activity as shown by the titres obtained with guinea-pig complement. No conclusions can, however, be drawn about binding *in vivo*, since human antibodies may fix human complement less well than complement from another species (Hoet, Blomfield & Coombs, 1949).

All eleven patients with anticomplementary sera had a clinical picture consistent with a circulating immune complex pathogenesis, and five of them were already known to have immune complexes that precipitated in the cold. In the remaining six, who did not have cryoglobulins, the anticomplementary activity could be interpreted as evidence that they, too, had circulating immune complexes.

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Localized secondary telangiectasia in patients with thyroid disease

JOHN A. THOMSON AND RONA M. MACKIE

University Department of Medicine, Royal Infirmary, and
University Department of Dermatology, Western Infirmary, Glasgow

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SUMMARY

The presence of telangiectatic lesions on the arms of patients who suffer from thyroid diseases is described and the histology of the lesion illustrated. The incidence of this abnormality was found to be 23% in patients attending a thyroid clinic as compared with 1% in patients attending a dermatological clinic.

The skin and its appendages shows characteristic changes in thyroid disease. In thyrotoxicosis it is characteristically warm with increased sweating; the nails may show onycholysis and the hair may be sparse. In hypothyroidism the skin is usually dry and coarse, except in those cases of hypothyroidism secondary to pituitary insufficiency, while loss of the scalp hair is characteristic and brittleness of the nails may be observed. These changes usually revert to normal with appropriate therapy (Freedberg, 1971).

Increased capillary fragility in thyrotoxicosis and decreased capillary fragility in hypothyroidism have also been described (Thomson, 1964). These also revert to normal with appropriate therapy.

The present study was prompted by the observation of an additional dermatological feature on the arms of patients attending a thyroid clinic.

CLINICAL MATERIAL

Certain patients attending the Thyroid Clinic at the Royal Infirmary, Glasgow, were noted to have red elevated lesions 1–3 mm in diameter on their arms, especially on the anterolateral aspect of the forearms and upper arms, but also in a small proportion on the back (Fig. 1). There was no history of application of topical steroids to these sites. On first examination the lesions were thought to be purpuric spots but closer inspection revealed blanching on diascopy.

Five cases were selected for detailed study, and the clinical details were as listed in Table 1. All patients were euthyroid at the time of examination. The length of time for which these lesions are recorded to have been present is in every case a minimum period, as no patient could state this with accuracy. The superficial appearances were those of multiple sharply demarcated circumscribed vascular lesions which blanched on pressure with no surrounding haemorrhage, purpura or visible draining capillaries. Biopsies were performed, and Fig. 2 shows the histology of a lesion stained for alkaline phosphatase using the Gomori method (Pearse, 1968). This shows a coil of dilated capillaries

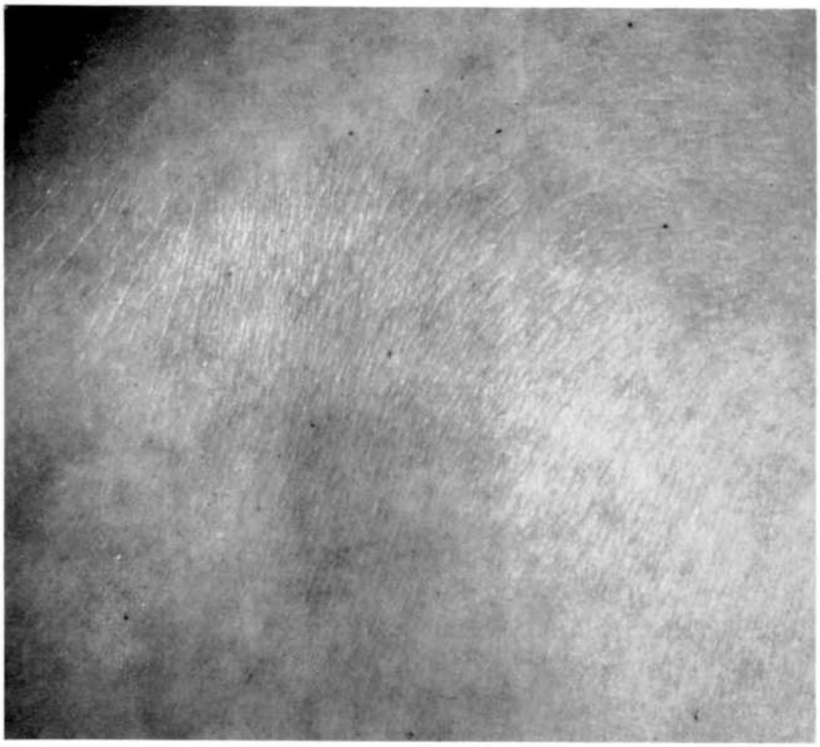

FIGURE 1. Forearm of case no. 2 showing multiple vascular lesions as dark dots.

lying in the papillary dermis. The overlying dermis is normal, there is no free haemorrhage round the lesion, and no inflammatory reaction. Alcian Blue stains were strongly positive for mucin, indicating the presence of myxoedematous tissue around the lesion.

The presence of these capillary dilatations was looked for in 100 successive patients attending the Thyroid Clinic. They were found in twenty-three cases of various thyroid diseases, including non-toxic goitre, hypothyroidism and treated thyrotoxicosis. Fifty additional patients with untreated thyrotoxicosis were examined before treatment and no such lesions were observed. The lesions were seen also in a few patients with treated hypothyroidism secondary to hypopituitarism. A similar

TABLE 1. Clinical details of five patients

Case no.	Sex	Age	Endocrine disorder	Site of lesions	Duration (years)	Therapy
1	F	67	Sheehan's syndrome	Forearms and upper arms	10	Sodium thyroxine 0·15 mg, cortisone 37·5 mg
2	M	64	Hypothyroidism	Forearms and back	8	Sodium thyroxine 0·4 mg
3	F	55	Hypothyroidism	Forearms	1½	Sodium thyroxine 0·2 mg
4	F	74	Hypothyroidism	Forearms	4	Sodium thyroxine 0·2 mg
5	F	30	Sheehan's syndrome	Forearms	3	Sodium thyroxine 0·15 mg, cortisone 50 mg

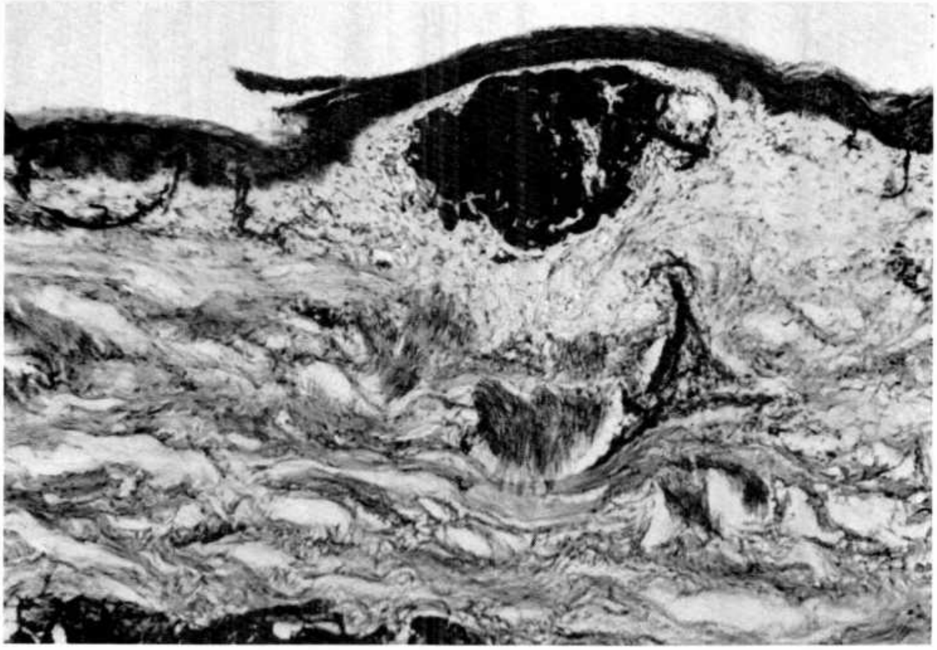

FIGURE 2. Lesion from case no. 2 stained by Gomori method for alkaline phosphatase. Normal capillary loops to the left contrast with the densely staining lesion.

search was carried out in 100 successive patients attending the dermatological out-patient department, and the lesions were observed in only one patient, a female attending for treatment of lichen planus. Clinically hypothyroidism was suspected, but laboratory tests carried out, including protein bound iodine and radio-iodine uptake, were within normal limits.

DISCUSSION

The lesions here described would seem to belong to the group of secondary telangiectasias. They are smaller than the average Campbell de Morgan spot, and have none of the surrounding network of small vessels associated with spider naevi.

In view of the high proportion of patients with thyroid diseases, excluding thyrotoxicosis, found to have these lesions, it seems remarkable that they have not been reported previously. The patients themselves have been aware of the lesions but have not sought advice, both because of the lack of symptoms and because of the small size of the lesions, which are not a cosmetic diability, even when present in relatively large numbers.

There would appear to be no regression of these lesions following treatment of hypothyroidism and return to the euthyroid state. Indeed in the majority of cases, the lesions have not been observed until the patient became euthyroid, but in view of the uncertainty over the time of appearance of the lesions expressed by many of the patients, this fact is somewhat doubtful. In view of the strongly positive Alcian Blue reaction seen around the lesion, it seems possible that lack of normal collagen support permits capillary dilatation, and that this is not a reversible phenomenon. Why this should occur preferentially on the arms is not apparent, but the preferential location of pretibial myxoedema on the lower leg is another indicator of the fact that the skin is not uniformly receptive to endocrine stimuli.

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Evaluation of skin damage. II

WATER LOSS AND CARBON DIOXIDE RELEASE MEASUREMENTS RELATED TO SKIN RESISTANCE MEASUREMENTS

K.E.MALTEN AND F.A.J.THIELE

Department of Dermatology, University of Nijmegen, Javastraat 104,
Nijmegen, The Netherlands

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SUMMARY

Water loss and carbon dioxide release measurements were performed to determine whether the severity of damage to human skin, caused by exposing its surface to buffered and unbuffered solutions of alkali and sodium phosphate, could be evaluated simultaneously by means of different measuring techniques and whether the results of these measurements, expressed in numerical terms, gave identical information.

It appeared that the buffered solutions disturbed skin functions less than the unbuffered solutions and therefore may be considered to be less harmful. The NaOH solution at pH 12·0, lacking any buffering capacity, damaged the skin, producing signs of inflammation, whereas that at pH 10·0 did not.

It appeared that skin damage could be assessed reliably by simultaneously measuring changes in skin impedance, water loss and carbon dioxide release, which are interrelated parameters.

Since *in vitro* experiments have shown a good correlation of impedance with water permeability (Allenby *et al.*, 1969) and since we have information available about the influence that chemicals may have on water loss *in vivo* (Thiele & Horst, 1967; Thiele & van Kempen, 1971), the results of our impedance measurements *in vivo* (Thiele & Malten, 1973) were compared with water loss and carbon dioxide release measurements performed on the same skin sites, *in vivo*.

METHODS AND APPARATUS

The water loss, carbon dioxide release and skin temperature were measured using methods previously described (Malten & Thiele, 1972; Schutter, 1970; Spruit & Malten, 1968; Thiele & Horst, 1967; Thiele & van Kempen, 1971, 1972; Thiele, Hemels & Malten, 1972; Thiele & Malten, 1972; Thiele & van Senden, 1968). These three parameters were measured previously to, and 24 hr after, each particular exposure.

The delta values for water loss (the value for the 'non-exposed' control skin site, exposed daily to a 0·09% NaCl solution, subtracted from the value measured on the experimentally 'exposed' skin site) and the calculated percentages (in which the initial water loss of the test site, or the simultaneously measured water loss of the control site was the 100% reference) both served as numerical terms for experimentally provoked skin damage.

All data are expressed as percentage alterations, thus simplifying comparisons.

The exposures were carried out as follows:

Left forearm	Right forearm	pH adjusted to
L_1 = NaCl	R_1 = Na_3PO_4 (pH 10)	10·0
L_2 = NaOH (pH 10)	R_2 = NaCl	—
L_3 = NaCl	R_3 = Na_3PO_4 (pH 12)	12·0
L_4 = NaOH (pH 12)	R_4 = NaCl	—

The combined and subsequent measurements of water loss, carbon dioxide loss and skin resistance involved two additional exposures to 0·09% NaCl daily during the 15 min previous to, and after each, specific exposure (see Discussion).

RESULTS

The effects of six sequential skin exposures to alkali and phosphate solutions (pH 10·0 or pH 12·0, 60 min daily) on water loss by skin are shown in Fig. 1. It can be seen that the pH 10·0 solutions hardly increased (NaOH) or slightly decreased (Na_3PO_4) the water losses. No inflammation was observed. However, the NaOH (pH 12) and Na_3PO_4 (pH 12) (adjusted to pH 12·0) caused increases in water loss of about 350%. The increase caused by the Na_3PO_4 (pH 12) was less than that due to the NaOH (pH 12) solution: the NaOH (pH 12) damage progressed faster. Recovery of water loss values progressed slowly: after 4 days the loss at the NaOH (pH 12) and Na_3PO_4 (pH 12) treated sites were still about 190% and 150% above normal respectively.

Each of the 60 min exposures to the pH 12·0 solutions caused marked inflammation. Sometimes vesicles were visible on the NaOH (pH 12) skin site (Thiele & Malten, 1973). The carbon dioxide release measurements were similar to the water loss data, although the maximum increase was restricted to about 120% (Fig. 2).

The magnitude of the effects of the solutions were in the following order: NaOH (pH 12) > Na_3PO_4 (pH 12) > NaOH (pH 10) > Na_3PO_4 (pH 10) (Table 1).

DISCUSSION

The data for water loss and carbon dioxide release have been compared with impedance data from the same skin sites (Table 2.) Skin impedance measurements (Thiele & Malten, 1973) revealed fairly similar information: skin resistance can be abolished almost completely by a 15–30 min exposure to NaOH (pH 12) solution, whereas exposures up to 60 min to NaOH (pH 10) solution hardly influenced skin resistance.

It is evident that the three techniques provide virtually identical information. Possibly the alkaline (pH 12·0) solutions increased water vapour and carbon dioxide diffusion through the horny layer, either by a physico-chemical interaction, causing a conformational collapse of the proteins of this layer and a consequent increase in porosity (Thiele, 1972; Thiele & Bauer, 1970), or by damaging the ductal epithelium of the sweat glands.

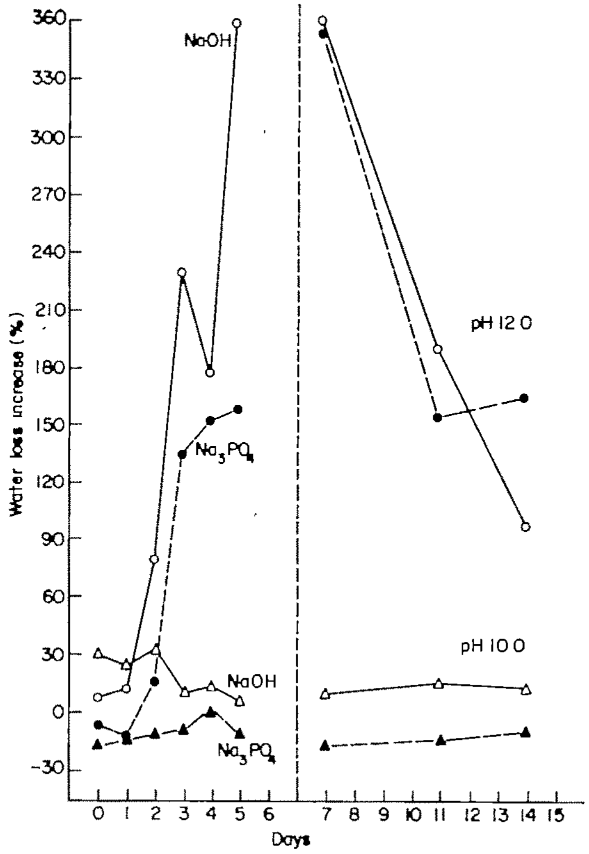

FIGURE 1. Water loss curves (percentage increase) (delta increases in water losses). The effects of six sequential 60 min exposures to NaOH and Na_3PO_4, adjusted to pH's of 10·0 and 12·0 respectively. No water loss measurement was carried out on Day 6 (Sunday). After 24 h (Day 7) a water loss measurement was carried out again: since impedance measurements were carried out before and after the six NaOH/Na_3PO_4 (pH 10 and 12) exposures, the skin sites were also exposed for 15 and 60 min to a 0·09% NaCl solution.

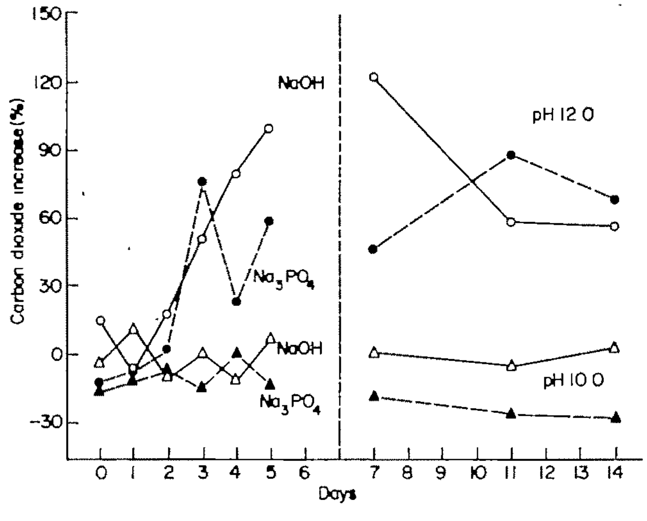

FIGURE 2. Carbon dioxide release curves (percentage increase). The effects of six sequential exposures (60 min) to NaOH and Na_3PO_4 adjusted to pH's of 10·0 and 12·0 respectively. Also see legend to Fig. 1.

TABLE 1. Percentage increases in water losses by skin after sequentional exposures to different alkaline test solutions at pH 10·0 and 12·0

24 hr after exposure no.	Chemical exposures	Impedance measurement	Exposure scheme 1			
			NaOH (pH 12)	Na_3PO_4 (pH 12)	NaOH (pH 10)	Na_3PO_4 (pH 10)
	×	+				
1	×	+	70	15	30	−15
2	×	+	220	135	10	−10
3	×	+	170	150	15	0
4	×	+	360	160	5	−15
5	×		*	*	*	*
6			360	350	10	−20

The percentage increase in water loss was calculated by subtracting the water loss value of a comparable skin site exposed to 0·09% NaCl solution (being the 100% reference) from the water loss value measured at the skin area exposed to the alkaline test solution. ×, first or continued exposure. *, no measurement carried out.

Frequent skin impedance measurements may perhaps exert some influence on the diffusional resistance of skin. However, the almost immediate abolition of skin impedance by a pH 12·0 solution (a 15–30 min exposure) suggests the establishment of a water-soaked contact brought about either via the two cell layers lining the sweat duct or via the wick of this duct, rather than by means of the unshielded multi-layer surface epithelium (the horny layer). On the other hand, the very short time that a dilute salt solution exposure (0·09%) needs for minimizing such a severe disturbance by NaOH (pH 12) points to a physico-chemical interaction between the alkali and the epithelial protein (Thiele, 1972; Thiele & Bauer, 1970), i.e. deprotonation of the horny layer proteins and as a consequence the transition from an ordered helix into a disordered random coil conformation, rather than to pathophysiological cell processes. The same should hold true not only for the ducts (perhaps the wick, lining the duct), but also for the sweat glands themselves. The conformational transition aforementioned can easily be achieved *in vitro* (Thiele, 1972) by adjusting the pH of a solution of purified

TABLE 2. Percentage increases in water loss, carbon dioxide release and decrease in skin resistance (all measurements were carried out 24 hours after an exposure) (see Fig. 1)

Measurements carried out on day no. (See Fig. 1)	Na_3PO_4 (pH 10·0)			Na_3PO_4 (pH 12·0)			NaOH (pH 10·0)			NaOH (pH 12·0)		
	R*	WL†	CO_2‡	R	WL	CO_2	R	WL	CO_2	R	WL	CO_2
2	5	−15	−10	100	15	2	25	30	−10	100	70	20
3	−60	−10	−15	100	135	75	55	10	0	100	220	50
4	−20	0	−0	100	150	28	50	15	−10	100	170	80
5	10	−15	−12	100	160	60	45	5	+5	100	360	100
6		×	×		×	×		×	×		×	×
7		−20	−20		350	50		10	0		360	125
Recovery Day 14 after first exp.		−10	−25		170	+75		15	+3		100	+60

*R, decrease in skin resistance (%); †WL, increase in water loss (%); ‡CO_2, increase in carbon dioxide release (%); ×, exposures continued.

horny layer protein to about 10·0, although *in vivo* this pH does not affect these proteins, perhaps because lipids protect the proteins of the horny layer structure. This protection can only be abolished by initially increasing the pH to about 12·0; thus the proteins will become accessible for the hydroxyl-ions of the alkaline solutions. It can be assumed that organic solvents, and perhaps detergents, will have a similar effect.

In addition to this, it can be argued that both sweat gland (epithelium and duct) and horny layer (surface epithelium) are involved in the interaction with alkali (pH 12·0), since we found that the alkaline solutions extracted about six times more amino acids and urea from the skin than did water alone: the amino acid composition of these extracts equalled the composition of separately collected pure sweat (Thiele & van Kempen, 1971).

Other methods could possibly provide more information about variations in function of the sweat glands as pathways for water vapour transport, and hence as contribution to the overall water vapour loss by skin (Thiele & Malten, 1972). We have therefore studied the sweating rate capacity of damaged and undamaged skin by thermal and pharmacological stimulation of the sweat glands. The results of these experiments will be published subsequently.

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Freeze-fracture replication of X-granules in cells of cutaneous lesions of histiocytosis-X (Letterer–Siwe disease)

A.S.BREATHNACH, M.GROSS, F.BASSET* AND C.NÈZELOF†

Department of Anatomy, St Mary's Hospital Medical School, London, W.2,
*Groupe de Recherches U 82 de l'I.N.S.E.R.M., Hôpital Bichat, Paris, and
†Groupe Hospitalier Necker-Enfants-Malades, Paris

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SUMMARY

Fresh, not chemically fixed, glycerol-cryoprotected material from skin lesions of histiocytosis-X (Letterer–Siwe disease) was examined by the freeze-fracture technique. Fracture appearances of the cytoplasmic X-granules characteristic of cells of histiocytosis-X were established, and the accuracy of previous three-dimensional reconstructions of variations, in overall shape of granules based upon stained thin sections was confirmed. These variations, therefore, are not artifacts of conventional tissue processing. Little or nothing of the internal structure of X-granules is seen in replicas, and the technique provides no new information concerning their origin or mode of formation.

It seems quite clear now that the technique of freeze-fracture replication has considerable potentialities for advancing knowledge of the functional morphology of biological tissues (Steere, 1957; Koehler, 1968; Branton, 1971; Moor, 1971). Apart altogether from its value in revealing intimate structural details of membranes and organelles it provides a new dimension for the examination of general ultrastructural topography, which can supplement the information derived from conventionally processed thin sections.

A major advantage of the technique is that tissues do not require to be chemically fixed, dehydrated, infiltrated with embedding media, or stained. They can therefore be examined in a condition which is close to the living state. Much of the pioneer work with this technique was carried out upon botanical specimens or on isolated cells and organelles of animal tissues. It is only comparatively recently that organized human tissues have been submitted to examination (Reed & Rothwell, 1970; Breathnach, Stolinski & Gross, 1972a; Scott McNutt, Herschberg and Weinstein, 1971), and attention to date has mainly been directed towards establishing normal appearances, and equating them with features revealed in chemically-fixed and stained thin sections. Pathological material is virtually unexplored, and it might be argued that it should remain so, until all normal features of a particular tissue as revealed by the technique have been documented. However, there are some pathological conditions characterized by the presence of cells of individual type, frequently with unique marker organelles rendering them readily identifiable, which it seems justified to investigate even at this early stage. Histocytosis-X is such a condition. The characteristic feature of the disease is infiltration of tissues (bone, lung, and skin) with large histiocytic-type cells of doubtful lineage, but which bear a remarkable resemblance to the Langerhans' cells of certain normal epithelia (Basset & Turiaf, 1965; Basset & Nèzelof, 1966; Gianotti, Caputo & Ranzi, 1968). Both cell types contain an apparently identical cytoplasmic organelle (Langerhans' granule, X-body), the origin and nature of which remains obscure (Breathnach, 1965; Breathnach & Wyllie, 1967; Wolff, 1972). Establishment of the significance of this organelle could throw further light upon the nature of histiocytosis-X, and upon the function of the normal Langerhans' cell. Its morphological features as seen in conventionally processed thin sections are well established (Figs. 2 and 3), and it seemed worthwhile, therefore, to examine it by the freeze-fracture technique, in order to determine to what extent particular features of the granule are represented in replicas. Freeze-fracture replication should also allow one to check the validity of three-dimensional reconstructions of granules based upon serial thin sections (Sagebiel & Reed, 1968; Tusques & Pradal, 1969), and to negate, or confirm, the suspicion that some of their bizarre shapes may result from conventional tissue processing. Owing to the relative scarcity of Langerhans' cells in normal epithelia, and the probability, therefore, that very few would be encountered in replicas, it was decided to initiate this study by an examination of cells of histiocytosis-X, which may be abundant in lesions, and which are known individually to contain many more of the characteristic organelles than do Langerhan's cells. The study could provide a basis for a future comparison with Langerhans' cells, and could also establish more general features of histiocytosis-X cells as revealed by the technique.

MATERIALS AND METHODS

Biopsy specimens were obtained from four patients with histiocytosis-X. Two had osseous lesions, and two suffered from a chronic generalized form of the disease with pulmonary, osseous, and prominent skin lesions (Letterer–Siwe disease). Each specimen was divided in two for thin sectioning and freeze-fracture replication.

Thin sections

Blocks were fixed in cold 0·1 M cacodylate buffered 3% or, 1·7% glutaraldehyde at pH 7·4 for 1 h and post-fixed for 30 min in cold 1% osmium tetroxide at pH 7·4. Routine dehydration, and embedding in Epon followed, and thin sections were stained on the grid with uranyl acetate and lead citrate. Sections were examined in a Siemens Elmiskop 101.

Freeze-fracture replicas

Fresh-non-chemically-fixed pieces of tissue were immersed for 30 min in 20% aqueous glycerol, and for a further 30 min in 40% glycerol, at 4°C. Glycerol serves as a cryoprotective agent. Specimens were then rapidly frozen in an isopentane bath, and transferred in a special chuck to the freeze-fracture apparatus. This latter comprised a special fracturing device designed in this laboratory (by C. Stolinski), and attached to a standard Genovac Vacuum Coating Unit. After evacuation of the apparatus, specimens were fractured, and replicas of the fracture surfaces were made by evaporating platinum onto them from an angle of 45°. A backing layer of carbon was then evaporated normally to the surface. Specimens were then removed from the vacuum chamber, and allowed to reach room temperature before procedure for separating replica from tissue was instituted. Separation of the two was effected by digesting the tissue away from the replica with hot (60°C) concentrated nitric acid. Replicas were then washed in water and mounted on small-hole copper or gold grids for examination. A Phillips EM300 electron microscope equipped with a goniometer stage was used in this investigation.

It should be emphasized that 'etching' (or more correctly, 'sublimation') of the specimens on which this report is based was not performed. This involves sublimation of ice by raising the temperature of the fractured specimen to −100°C for several minutes before shadowing and replication. It is only applicable to specimens suspended in water or to organized tissue pre-treated with chemical fixatives before freezing. It is not applicable to non-chemically-fixed organized tissue treated with glycerol as cryoprotective agent, since significant sublimation does not occur under this condition. Chemical fixation leads to loss of fine detail, and, in fact, vitiates one of the major advantages of the technique, and sublimation can produce artifacts which complicate interpretation (Moor, 1971; Staehelin & Bertaud, 1971; Lickfeld, Achterrath & Hentrick, 1972). Attempts to produce satisfactory replicas of sublimated specimens using DMSO as cryoprotective agent, though encouraging, were not successful.

Micrographs of replicas presented in this report are all of skin specimens, since, confirming previous experience, many cells of the osseous and pulmonary lesions were necrotic.

In order to interpret the appearances revealed in micrographs of freeze-fracture replicas, it is necessary to appreciate what happens as the fracture traverses a frozen cell. It traverses the general cytoplasmic matrix in an apparently random fashion, but, as it approaches membranes (plasma, nuclear, or those surrounding organelles) it is frequently deviated, and runs parallel with the membrane for variable distances thus exposing extensive areas *en face*. It was thought originally that these *membrane fracture faces*, which carry variable numbers of particles—membrane-associated particles—represented true outer and inner surfaces of membranes. However, it is now generally agreed that the fracture traverses the interior of the membrane, and that the fracture faces revealed are internal or 'split' faces of the membrane (Chalcroft & Bullivant, 1970; Scott McNutt & Weinstein, 1970; Branton, 1971). It is clear that for any membrane that is fractured, one face will be present on each surface of the fractured tissue, i.e. the one that is fractured away, and the one that is replicated; these 'matched' faces, if brought together again, would reconstitute an intact membrane. If one considers, for instance, a membrane limited cytoplasmic organelle, and bears in mind that the plane of fracture lies within the membrane, then it is clear that the fracture can reveal two faces of the membrane, one (Ex) directed towards the exterior of the organelle, and another (In) directed towards its interior. Where a single organelle is concerned, only one or other of these faces will be represented on a given replica, the other being present on the tissue which was fractured away. However, where a number of similar organelles are present within a tissue, e.g. mitochondria, both types of face (each from a different organelle) are frequently seen on the same replica (Fig. 1). Such faces are referred to as 'complementary faces'. In a situation, such as an intercellular contact, where closely apposed membranes are separated by a narrow interval containing amorphous material, complementary fracture faces (one face of the membrane of each cell) are revealed, separated by an intercellular step (Breathnach *et al.*, 1972a). Membranes, of course, may also be fractured straight across, appearing therefore 'edge-on' in replicas, just as in thin sections.

RESULTS

Stained thin sections

Fig. 2 illustrates general features of a typical histiocytosis (H-X) cell, as well as the two-dimensional appearances presented by a sectioned X-granule. Exactly similar appearances are presented by the granules of the Langerhans' cell (Fig. 3). An image of the granule frequently seen in electron micrographs is that of a membrane-limited rod-shaped structure with a central linear density exhibiting periodicity. Wolff (1967) succeeded in resolving this central line into two closely set rows of particles with a spacing of 6–7 nm. He also demonstrated a row of particles with similar spacing just internal to the limiting membrane. On tangential sectioning of the granule a two-dimensional square

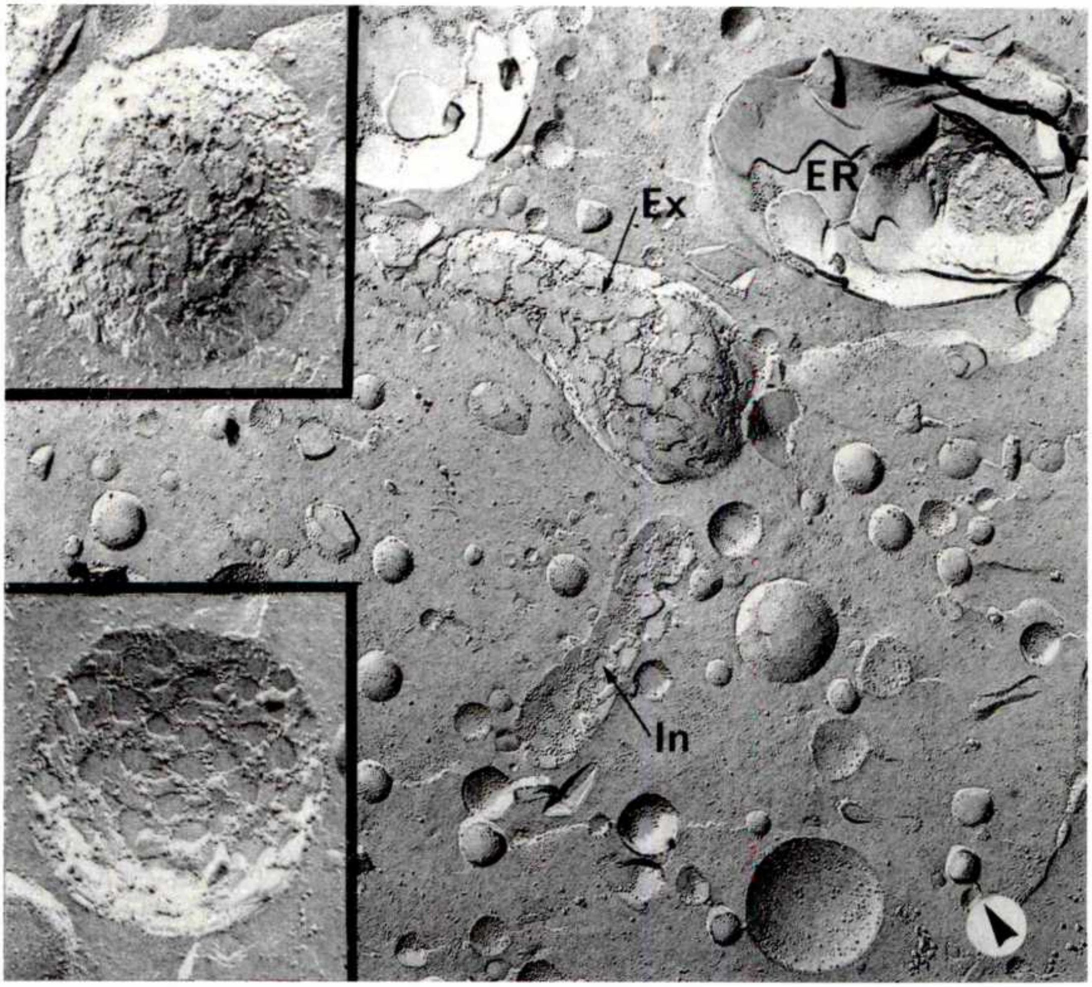

FIGURE 1. Freeze-fracture replica (of mouse liver cell) showing faces of mitochondrial membrane exposed during fracture. Ex, fracture face of membrane, directed towards the exterior of the organelle; In, fracture face of membrane directed towards interior of organelle. These faces are revealed by passage of the fracture within the membrane. They are 'complementary' faces in the sense that, though derived from two different mitochondria, they would, if joined together, reconstitute an intact membrane. Similar faces for each of the two mitochondria have been fractured away. ER, fracture face of membrane of rough endoplasmic reticulum (× 9000). Insets: similar complementary Ex and In faces from two other mitochondria (× 63,000). In this and succeeding micrographs, the large arrow at lower right indicates direction of platinum shadowing.

lattice of particles is frequently seen, which presumably represents one or other, or all, of the rows of particles referred to in the previous sentences. Blowing-out or vesiculation of the limiting membrane of the granule may frequently be seen (Fig. 3). By analyzing serial sections Sagebiel & Reed (1968) and Tusques & Pradal (1969) constructed three-dimensional models which showed that the granules may be disc-shaped, cup-shaped, or a combination of both shapes, with or without vesicular pouching of the limiting membrane, usually towards one end.

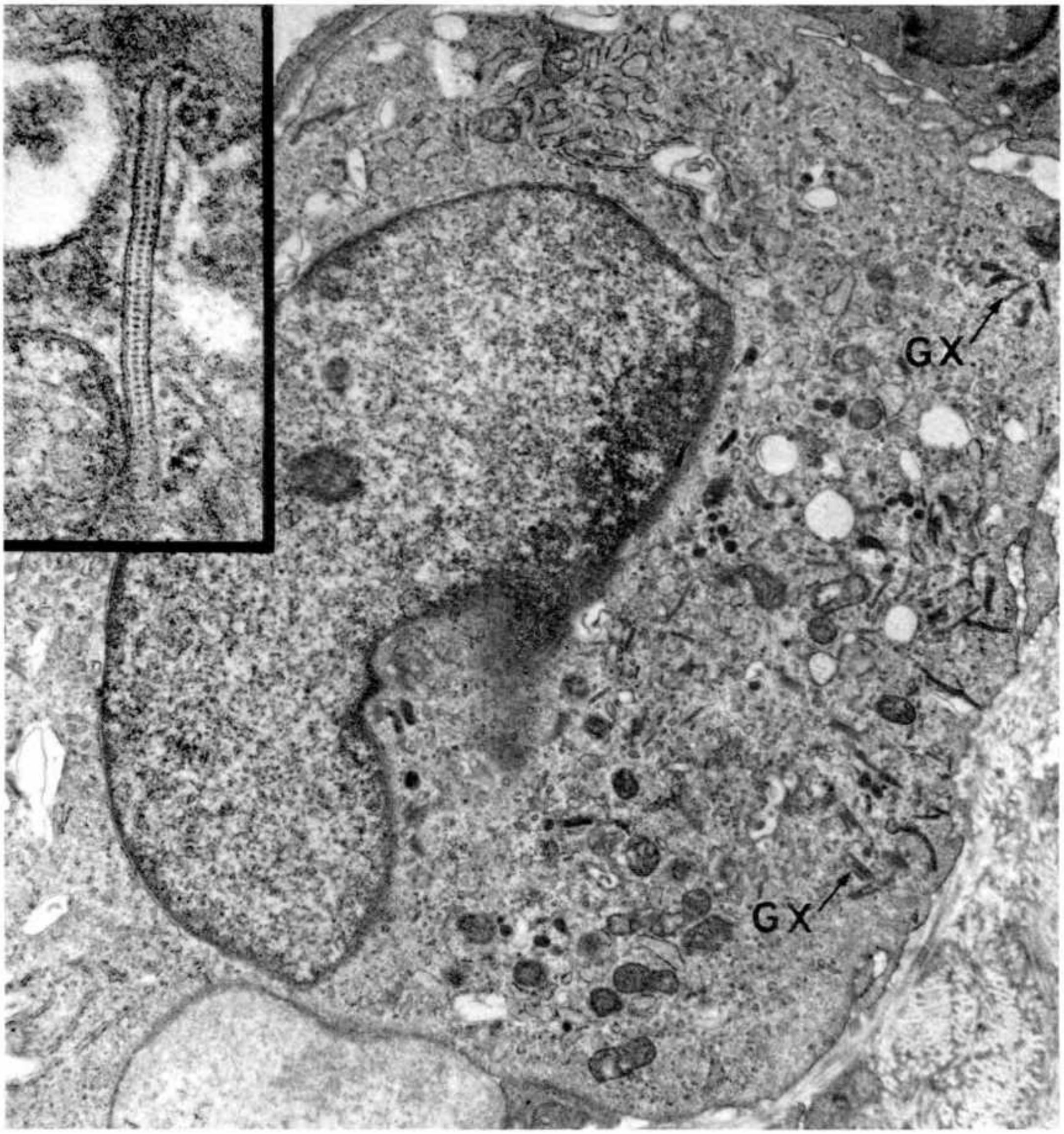

FIGURE 2. Stained thin section of cell typical of lesions of histiocytosis-X. Note X-granules (GX) in cytoplasm (× 11,500). Inset: typical sectional profile of X-granule. Note central periodicity, and row of particles just internal to the limiting membrane (× 108,000).

Freeze-fracture replicas

General features of X-cells. Fig. 4 is a survey micrograph of a replica of the cytoplasm of an X-cell for comparison with the thin sections. The majority of the organelles appear as rounded concavities or convexities. At higher magnification, some of these can be identified as fracture faces of mitochondrial membranes (Figs. 6 and 7), but it is not possible to be certain about others; they may represent lysosomes or other vesicular structures. Mitochondria fractured transversely, and fracture faces of the

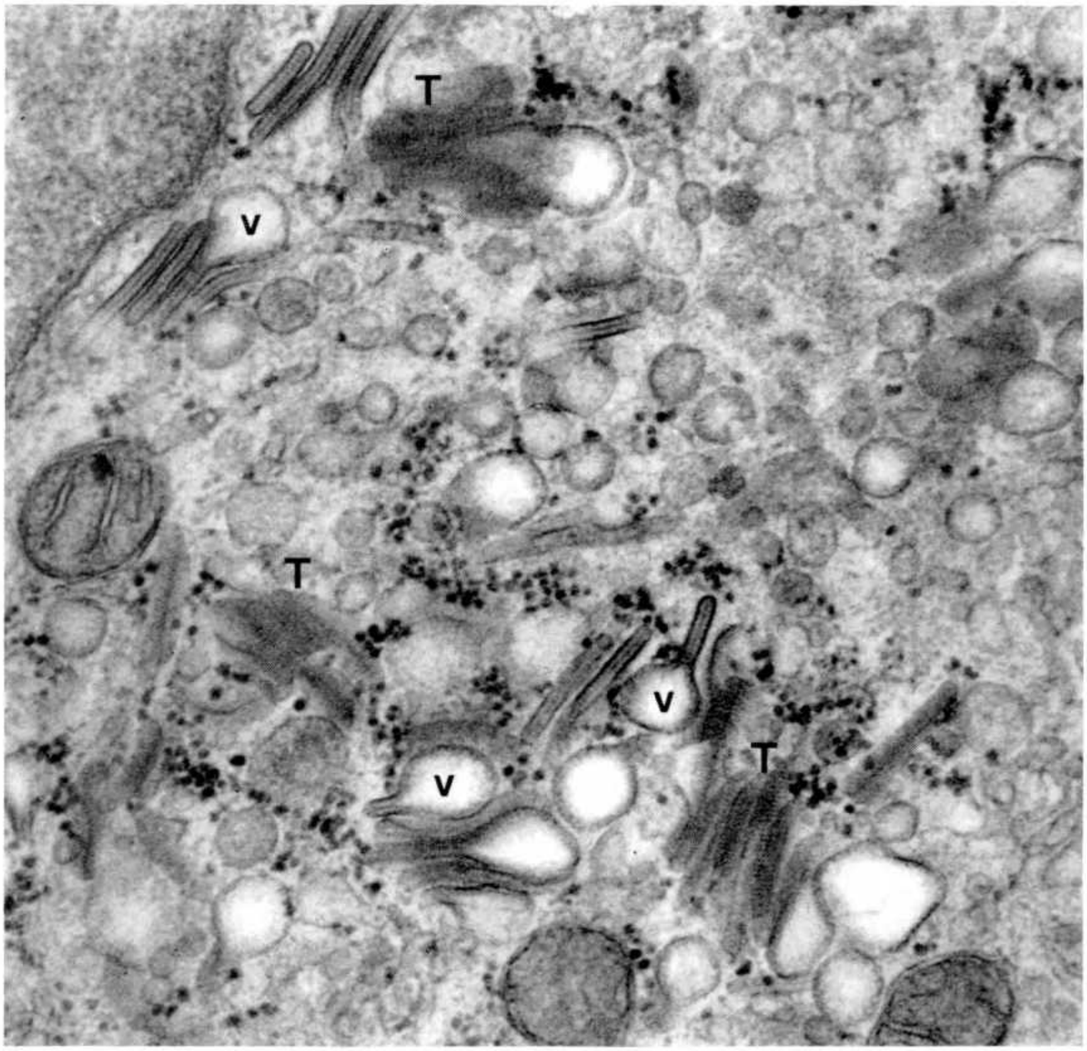

FIGURE 3. Stained thin section of cytoplasm of epidermal Langerhans' cell showing typical granules which are identical with X-granules of histiocytosis-X cells. Many of the granules show vesiculation (V) of the limiting membrane, and some have been sectioned tangentially (T) (× 68,000).

membranous component of the rough endoplasmic reticulum (Fig. 7), as well as Golgi membranes are also seen. The X-granules present a variety of appearances.

X-granules. Appearances clearly reminiscent of the rod-shaped profiles of thin sections are frequently seen in replicas (GX, Fig. 5). These are evidently X-granules which have been fractured straight across, and since, therefore, the limiting membrane lies practically flush both with the surrounding cytoplasm and the substance forming the interior of the granule, it may not always be clearly defined. These cross-fractured granules likewise exhibit little or no internal structure. It is only very occasionally that a suggestion of a row of particles just internal to the limiting membrane is evident (inset, Fig. 5), and a central linear density exhibiting periodicity is not perceived. In only one instance (Fig. 10) has anything been observed which remotely could be taken to represent this feature.

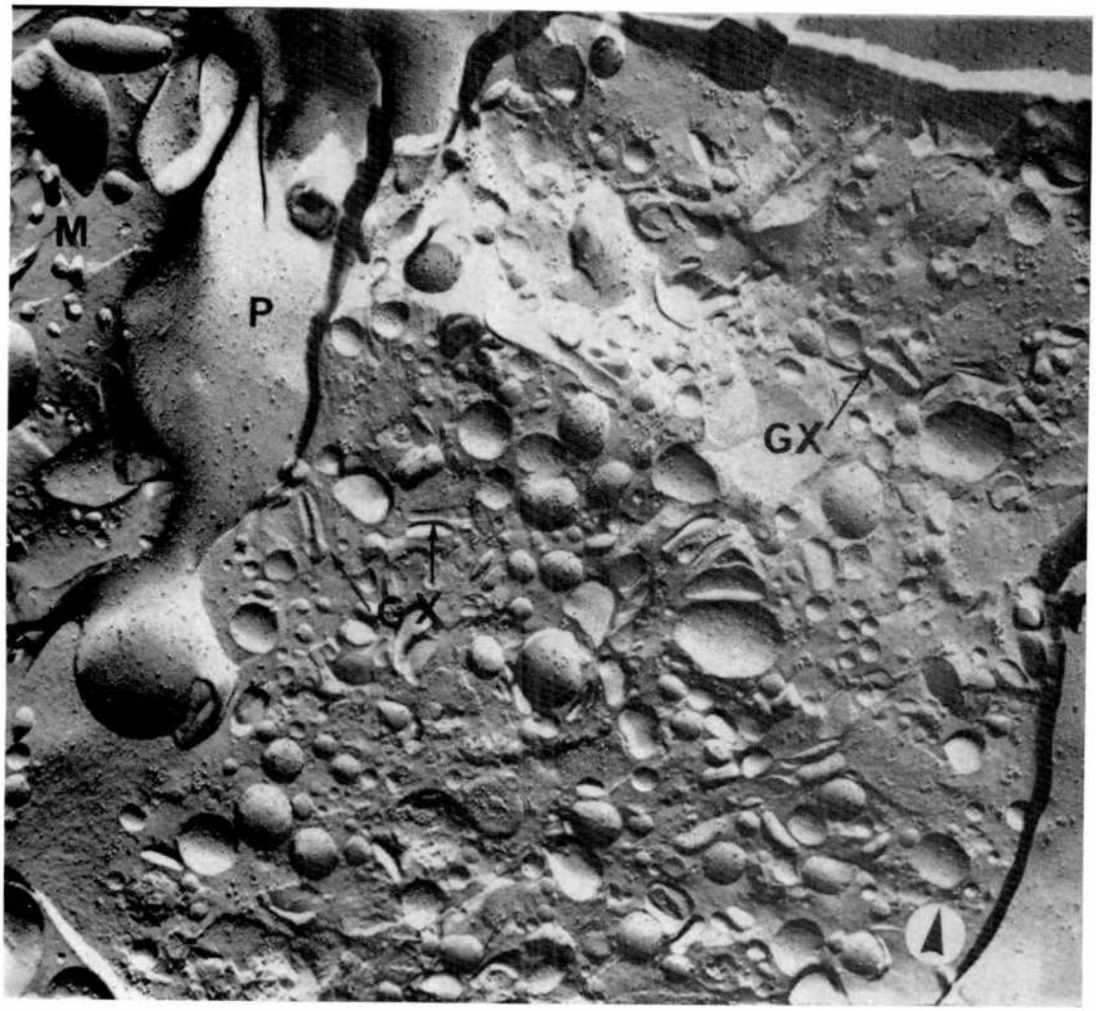

FIGURE 4. Freeze-fracture replica of cytoplasm of cell from skin lesion of histiocytosis-X. M, surrounding milieu; P, fracture face of plasma membrane. The cytoplasm of the cell contains many organelles which it is difficult to identify at this magnification, but some X-granules (GX) can be seen (× 30,600). Compare with Figs. 2 and 3.

Some rod-shaped structures, also evidently X-granules, appear (due account being taken of the direction of shadowing) slightly concave (GX_2, Figs. 5 and 6) or, less frequently, convex (GX_1, Fig. 6). Exactly what these represent will be considered below.

Single, or stacked elements (GX_3, Fig. 6) exhibiting two fracture faces separated by a step are also frequently seen. Again taking due account of the direction of shadowing, it is evident that one of these fracture faces (Ex) is directed towards the exterior of the structure concerned, and the other one (In) towards its interior—represented by the step. This appearance of two fracture faces separated by a narrow step is very similar to that encountered at appositions of epithelial cell plasma membranes (Breathnach *et al.*, 1972a) and is what one might reasonably expect to result from fracture of a flattened or disc-shaped, membrane limited, cytoplasmic organelle such as the X-granule. Accepting the now generally agreed view that the fracture traverses the interior of membranes, the two faces

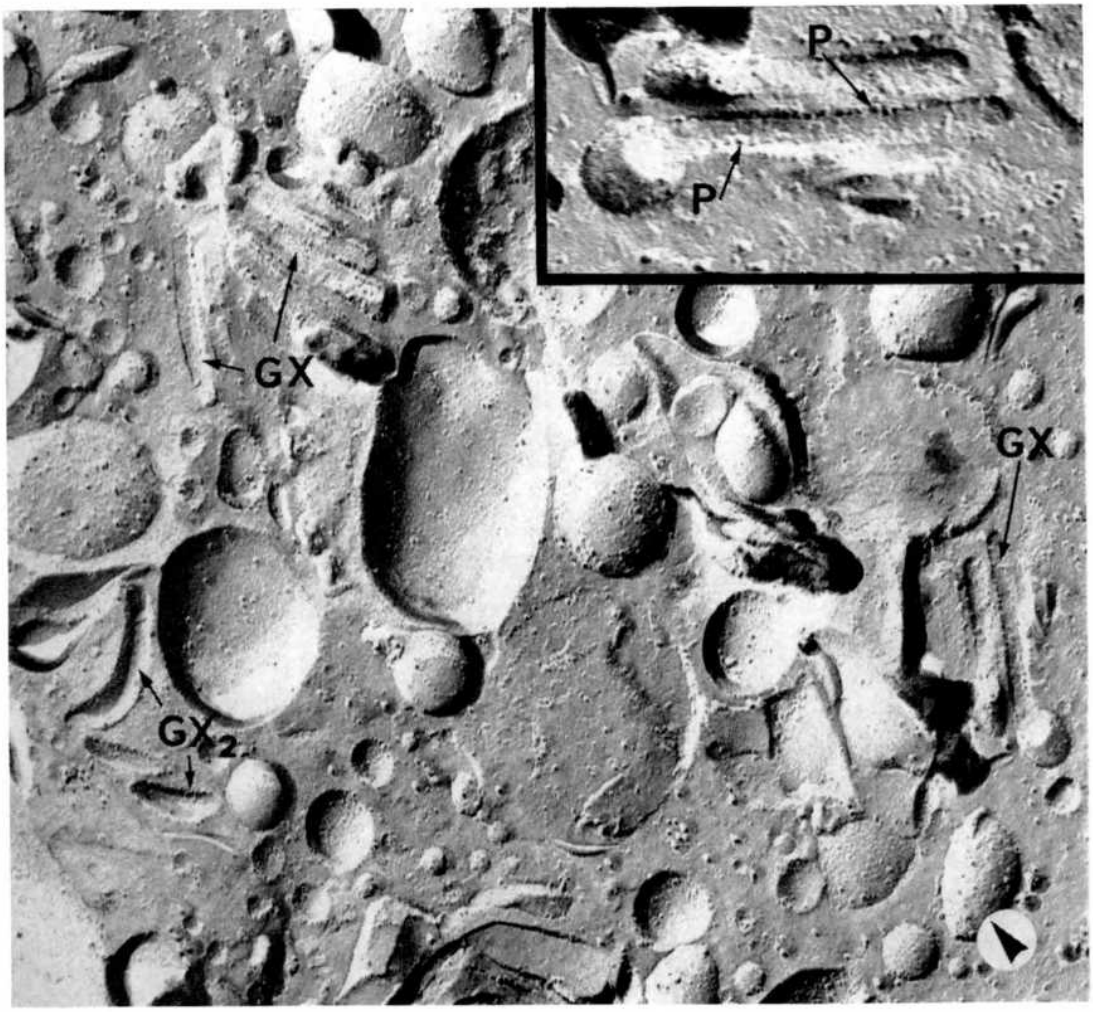

FIGURE 5. Freeze-fracture replica of cytoplasm of cell from skin lesion of histiocytosis-X. This micrograph shows disc-shaped X-granules (GX) fractured straight across. In this circumstance, fracture faces of the limiting membrane are not revealed, and the granules appear very similar to those seen in thin sections (cf. Fig. 3). GX_2, 'concave' (In) fracture face resulting from passage of fracture within extreme periphery of the membrane of a disc-shaped granule (× 64,500). Inset: cross-fractured X granule. Apart from a suggestion of a row of particles (P) just internal to the limiting membranes on each side, little of the internal structure of the granule as seen in thin sections is revealed (× 119,000).

concerned (Ex and In), must represent complementary fracture faces of the limiting membrane. As can be seen from Figs. 7 and 8, fracture face Ex carries more particles than fracture face In.

Having, as above, established the fracture appearances of essentially disc-shaped granules, it is then possible to identify fractured granules of more bizarre shape. Thus, in Figs. 9 and 10 can be seen; (1) what is evidently a discoid granule bent practically double with slight terminal distension of the limiting membrane, (2) disc-shaped granules indented or apparently impressed from one side, and (3) an area of membrane fracture face from the angles of which extend typical rod-shaped structures.

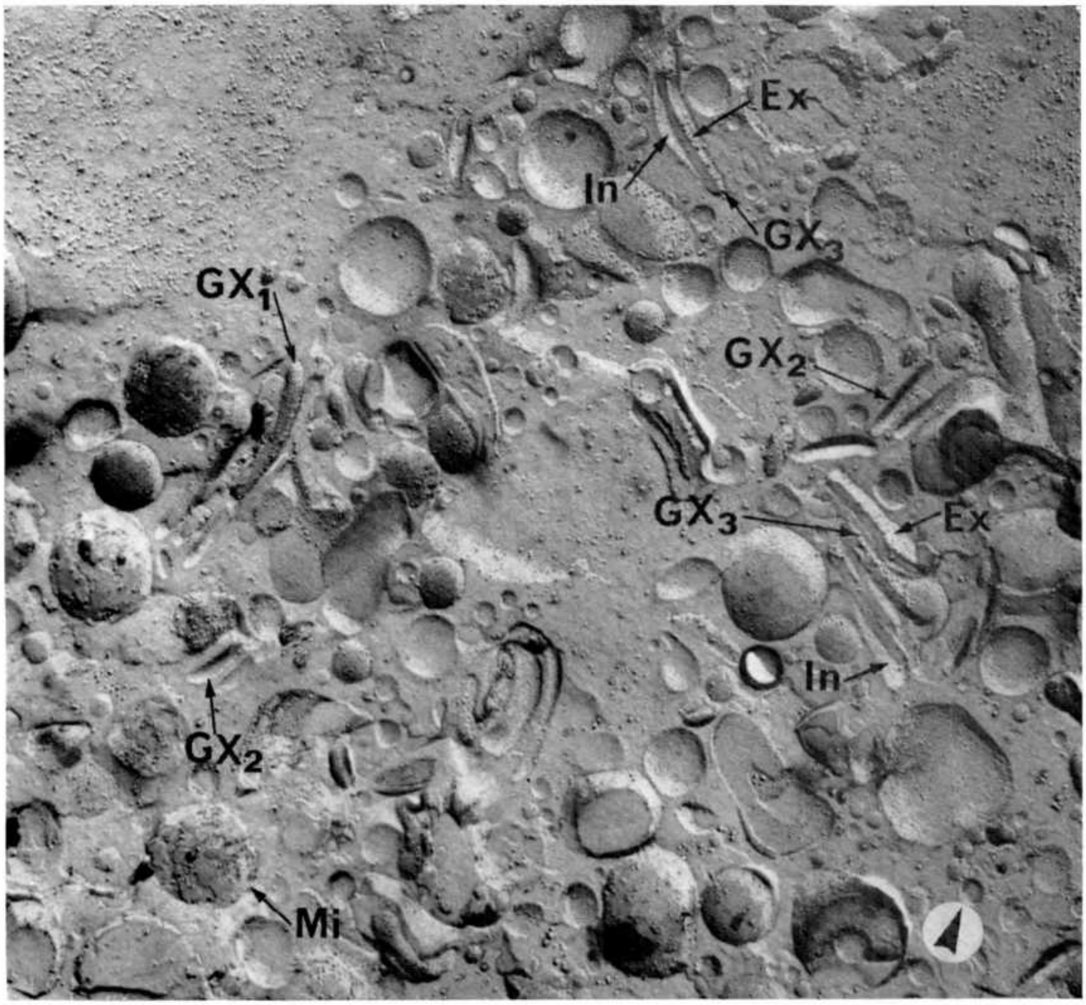

FIGURE 6. Freeze-fracture replica of cytoplasm of cell from skin lesion of histiocytosis-X. Some granules (GX_3) have been fractured so that complementary fracture faces of the limiting membrane (Ex and In) are revealed. See also subsequent micrographs. GX_1, GX_2, 'convex' (Ex) and 'concave' (In) fracture faces of extreme periphery of limiting membrane of disc-shaped granule. Mi, mitochondrion (× 50,000).

All of these appearances may be seen in stained thin sections. In many replicas (Figs. 8, 9, and 10) isolated fracture faces carrying practically no particles are seen. These are unlike any faces resulting from fracture of the common cytoplasmic organelles, and resemble closely in appearance fracture face In of the X-granule. Since they are not associated with the complementary fracture face Ex, it may be concluded that they result from fracture of a blown-out, or vesicated area of a granule, the fracture traversing the membrane, but not entering the internal substance of the granule. If this interpretation be correct, similar isolated areas of the complementary fracture face Ex should be seen in replicas. Certainly, many areas of suitable size and appearance are seen, but it is impossible to be certain about their identification, since these could also represent similar type fracture faces of other vesicular organelles. The concave and convex rod-shaped structures previously referred to (see Figs. 5 and 6)

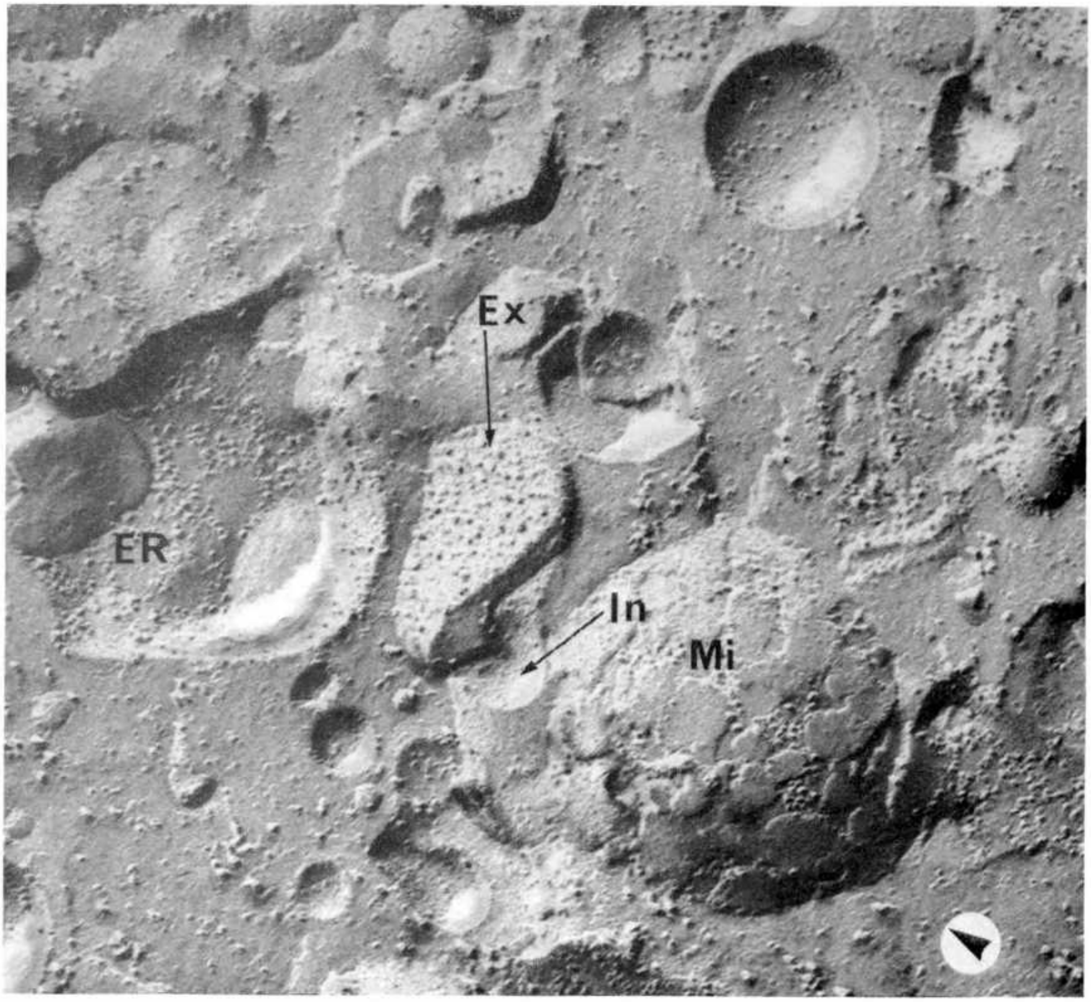

FIGURE 7. Replica showing fracture faces Ex and In of limiting membrane of X-granule. The two faces are separated by a step which represents the internal substance of the granule. Face Ex carries more particles than face In. Mi, mitochondrion; ER, rough endoplasmic reticulum (× 87,000).

almost certainly represent isolated complementary fracture faces In and Ex respectively, revealed by the fracture traversing the extreme periphery of the limiting membrane of a disc-shaped granule, without entering into the internal substance.

DISCUSSION

This study has established the freeze-fracture appearances of the cytoplasmic X-granules characteristic of cells of histiocytosis-X. The added three-dimensional effect provided by the technique reveals that previous reconstructions of variations in overall shapes of X-granules (Sagebiel & Reed, 1968; Tusques & Pradal, 1969) are accurate. Particular features of granules seen in thin sections are also

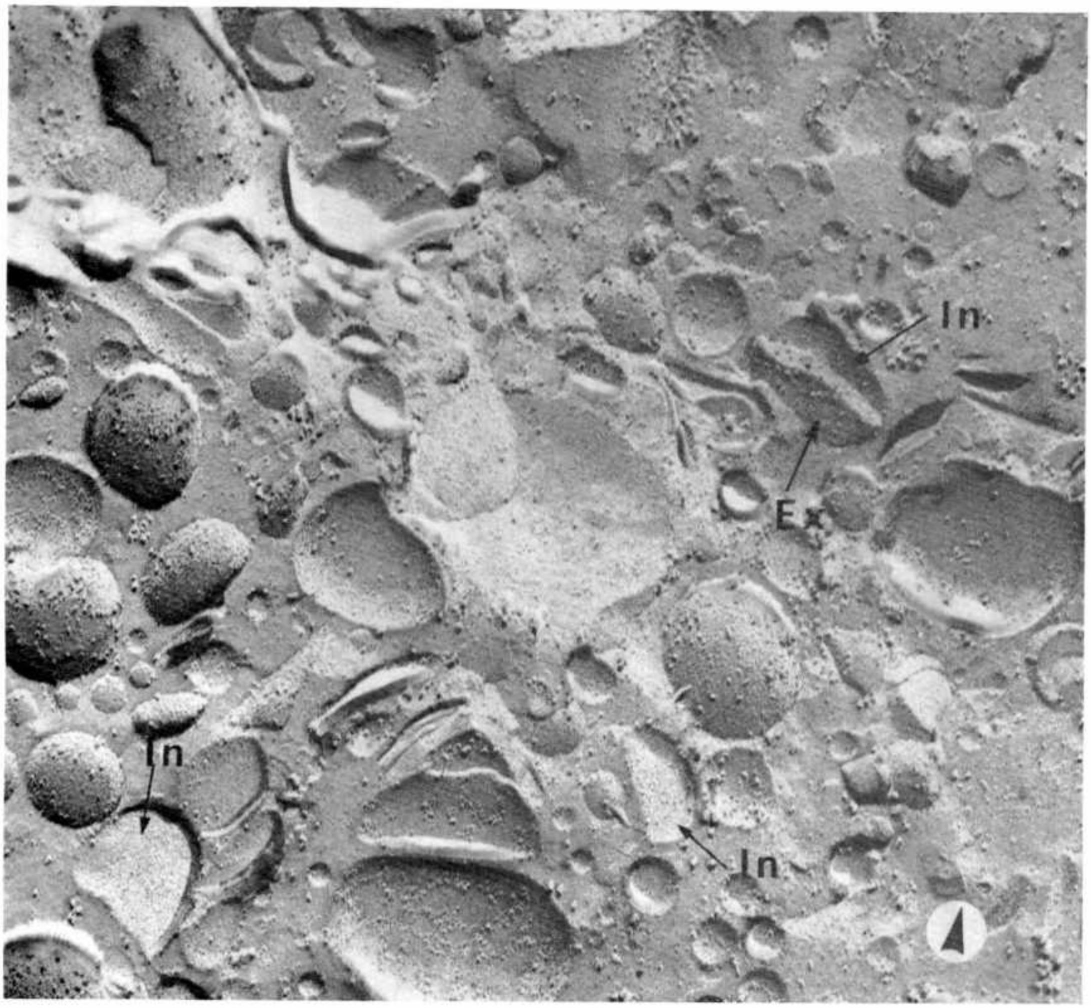

FIGURE 8. In this replica another fractured, disc-shaped granule exhibiting fracture faces Ex and In is seen. Towards the lower end of the field fracture faces of type In, unassociated with the complementary face type Ex, are seen. These are faces revealed by fracture of a blown-out or vesicated segment of a granule (× 68,000).

apparent in replicas of fresh unprocessed tissue, and therefore cannot be attributed to the effects of conventional tissue processing.

For the purpose of considering the path of the fracture in relation to the X-granule, and what it reveals, the disc-shaped granule may be likened to a sandwich, with the limiting membrane representing two slices of bread, and the internal substance of the granule the filling or meat of the sandwich. For any single granule, two faces of the limiting membrane (one for each 'bread slice', so to speak) are revealed (Figs. 6, 7, and 8) separated by a step which includes the internal material. These are complementary fracture faces of the membrane. This is essentially the same picture as that revealed on fracture of closely apposed plasma membranes of cells separated by a narrow interval (Breathnach *et al.*, 1972a). Of the two faces, the one directed towards the exterior of the granule (Ex) carries more particles than the complementary face (In) directed towards its interior. A similar disparity in numbers of

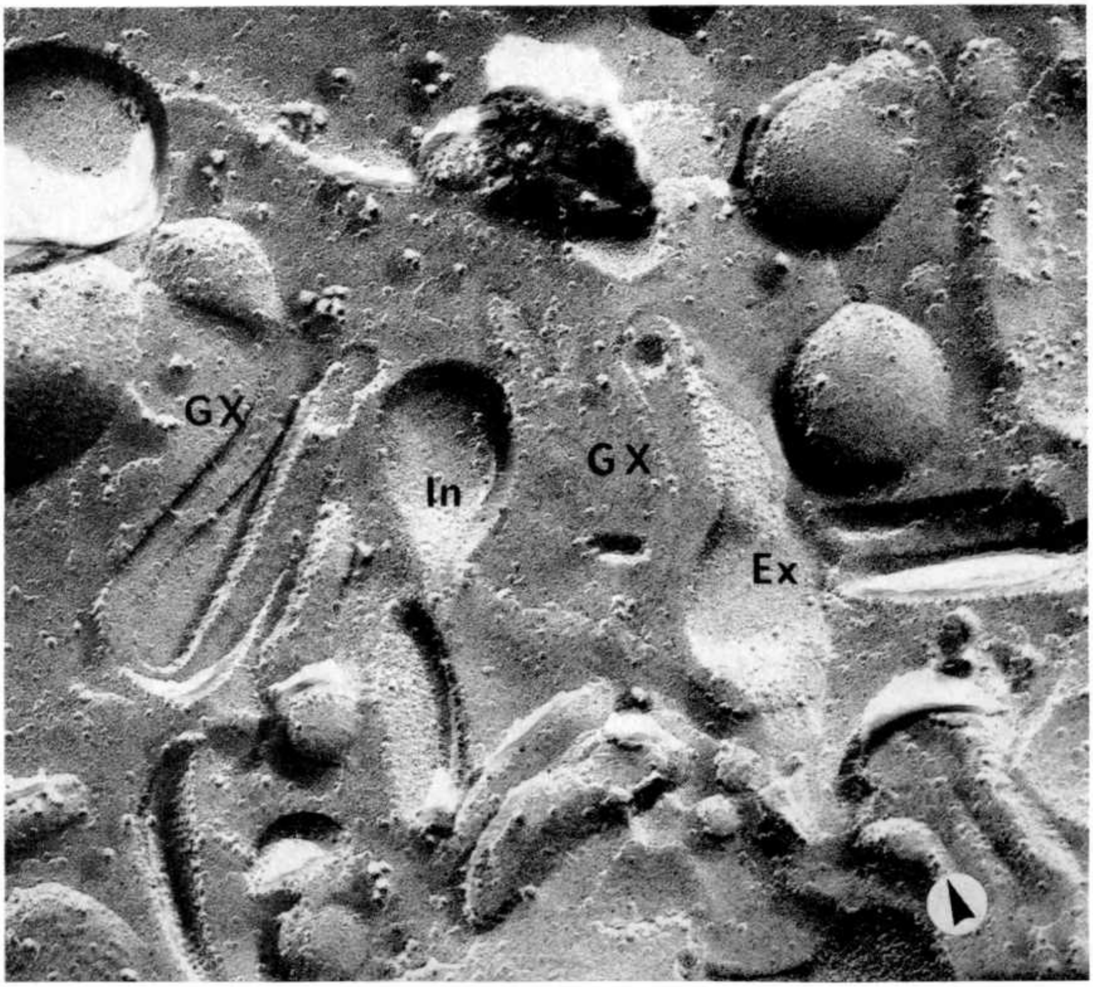

FIGURE 9. Replica showing X-granules (GX) of different shapes: (1) a cross-fractured granule bent almost double and with some distension of the limiting membrane at one end; (2) a disc-shaped granule exhibiting 'cupping' or indentation at one end. The fracture has revealed face Ex of one side of the limiting membrane, but the other side of the membrane has been fractured straight across and is only just apparent. In, isolated fracture face resulting from passage of fracture within vesicated segment of limiting membrane of an X-granule (× 123,000).

'membrane associated particles' is observed in relation to complementary fracture faces of plasma membrane. Fracture faces of the nuclear membrane, and of the membranous component of the rough endoplasmic reticulum and of mitochondria, display somewhat different features, of which a mosaic arrangement of particles and smooth areas is a significant feature (Breathnach *et al.*, 1972b). Since the fracture reveals the interior of membranes, these differences must have some basic structural or functional significance. The similarity between the fracture faces of the X-granule and those of plasma membrane could, therefore, be quoted in support of the suggestion that the granules are produced by infolding and 'nipping-off' of segments of plasma membrane. However, though observations are limited, it would appear that fracture faces of essentially similar appearance are revealed on fracture of

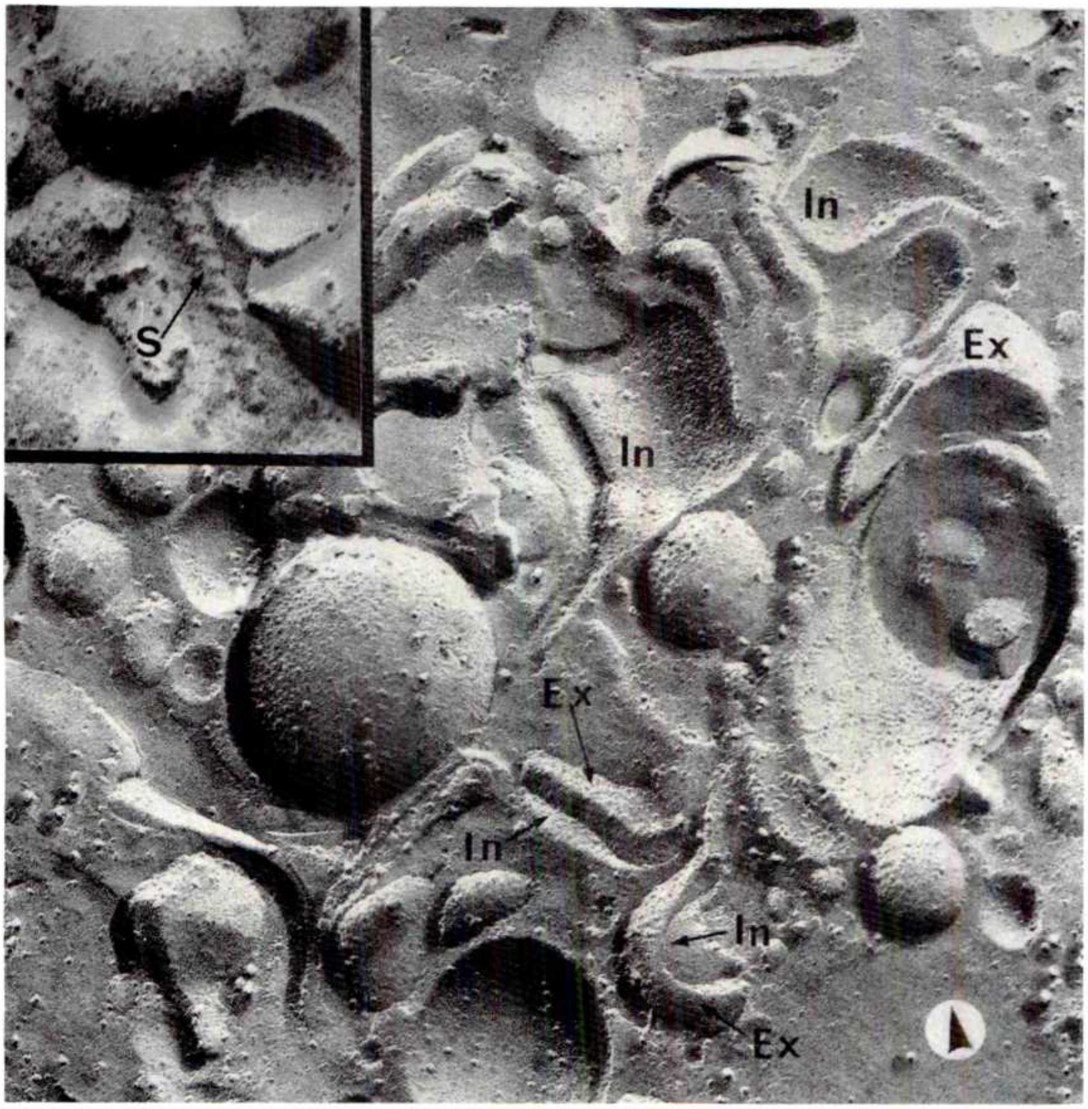

FIGURE 10. Replica from skin lesion of histiocytosis-X showing a variety of fracture appearances of X-granules. Towards the bottom of the field is a partially indented granule exhibiting fracture faces In and Ex, and above this a disc-shaped granule exhibiting similar faces. In the centre of the field is an area of fracture face type In with rod-shaped extensions from its angles. At top right, In and Ex faces of vesicated segments of granules (× 85,600). Inset: granule distended towards one end, but exhibiting apparent striation (S) in the non-distended part, which may or may not reflect periodicity seen in thin sections (see Fig. 2, inset) (× 176,000).

Golgi membranes, and this could equally well be quoted in favour of a Golgi origin for the granules. One must conclude that freeze-fracture contributes little towards solution of the problem of the origin of the granules.

The fact that freeze-fracture reveals little or nothing of the internal structure of the granule is not really surprising. There is no interior membranous component which would lead to deviation of the

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fracture, and so it traverses the material confined within the limiting membrane in a manner similar to its traverse of the general cytoplasmic matrix. If, or when, effective sublimation of unfixed, suitably cryoprotected material becomes a practical proposition, however, more information on this point should become available.

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An exaggerated erythema response to a cold pressure test in patients with cold urticaria

J.S.PASRICHA AND K.C.NAYYAR

Department of Dermatology and Venereology, All India Institute of Medical Sciences,
New Delhi-16, India

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SUMMARY

Conventional methods of testing patients with cold urticaria are unsatisfactory. In this report we have described an instrument which exposes a circular area of skin 33 mm in diameter to 0°C for 2 min. The response is recorded in terms of the maximum diameter of erythema which follows and of the duration for which this erythema persists. The flexor surface of the forearm was selected as a convenient site, and the responses from the two forearms were found to be almost equal. The response in most of the fifty normal controls and in twenty-four patients with urticaria of unknown origin consisted of an erythema less than 38 mm in its maximum diameter, lasting for less than 3 min. In twenty-eight of thirty-two patients clinically thought to have cold urticaria the response was exaggerated, either in the extent of erythema or in its duration or in both. Only one patient developed a weal. This test is thus considered to be useful in confirming the diagnosis of cold urticaria. The incidence of false positive and false negative reactions is quite low and can be minimized by the exclusion of certain factors which influence the test.

Some patients with urticaria develop their attacks on exposure to cold stimuli, such as a cold wind, a cold bath or cooled drinks, but testing these individuals with ice cubes (Sherman & Seebohm, 1950; Muller, 1961; Juhlin & Shelley, 1961; Fitzpatrick, 1963; Shafer, 1965) or by dipping their hands in cold water (Duke, 1924; Horton, Brown & Roth, 1936; Muller, 1961; Juhlin & Shelley, 1961;) induces urticaria in only a few. Attempts to demonstrate cryoproteins by the refrigeration test are as a rule negative. The passive transfer test (Houser *et al.*, 1970) for the demonstration of antibodies has not been tried by us for fear of serum hepatitis. Thus, many cases, although clinically very suggestive of cold urticaria, remain unconfirmed. The thermo-stimulator (Pasricha, Kandhari & Malhotra, 1969) was designed by us to standardize the cold stimulus to the skin in order to record small variations in the response of different individuals. We have used this instrument to establish whether patients with cold urticaria react differently compared to normal persons and to patients with urticaria due to other causes.

THE INSTRUMENT

Thermo-stimulator II (Fig. 1) is an improved version of the one described earlier (Pasricha *et al.*, 1969) and consists of an inner aluminium tube (I) 33 mm in its external diameter. The lower end of this tube is closed and constitutes the exposure surface (E) which is applied to the skin while testing.

The upper end of this tube is covered by a removable cap (C) which is perforated in the centre. Through this perforation a thermometer (T), which records the temperature of the exposure surface from within is passed into the inner tube. The main length of the inner tube, except for 5 mm adjoining the exposure surface, is surrounded by an outer tube (O) of a larger diameter. The space between the two tubes is filled with a material of low heat conductivity, and sealed. The outer tube is covered by a wooden shell (W) to further retard the exchange of heat with the environment.

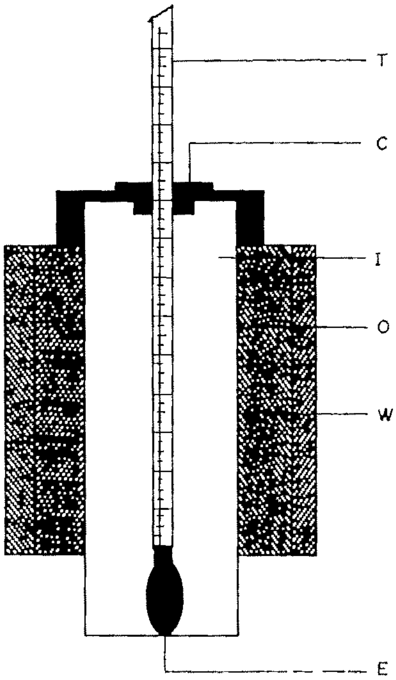

FIGURE 1. Diagrammatic longitudinal section of the instrument.

THE TEST

To perform the test (hereafter referred to as the cryo-stimulation test) the cap with the thermometer is removed and the inner tube filled to the brim with crushed ice. The cap and the thermometer are replaced and the temperature is adjusted to 0°C. The exposure surface is wiped dry and the instrument placed on the skin in such a manner that only the exposure surface touches the skin under the pressure of its own weight (300 g). After 2 min the instrument is removed and the reaction of the stimulated skin recorded.

The stimulated area shows an initial vasoconstriction. This is soon followed by a vasodilatation which spreads irregularly beyond the stimulated area. After a variable period the reaction subsides completely.

From experience with the responses obtained in several tests it has been decided to record the response in terms of: (1) maximum diameter of erythema developed during the responses, (2) duration for which the erythema persists, and (3) appearance of any weal.

STANDARDIZATION

Regional variations

In five normal subjects, the cryo-stimulation test was performed at various sites on the body, i.e. forehead, flexor surfaces of forearms, medial aspects of upper arms, backs of legs, fronts of thighs, infraclavicular regions of upper chest and peri-umbilical region of abdomen. Differences in the responses obtained from different regions were quite small. As a matter of convenience, therefore, the flexor surface of the forearm was selected for all subsequent tests.

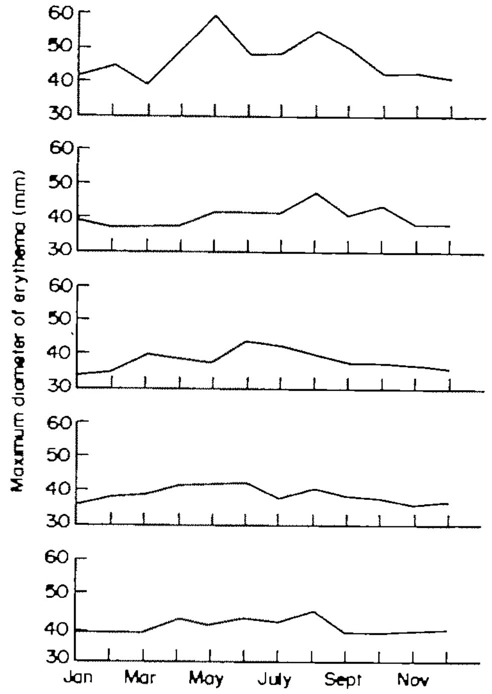

FIGURE 2. Variations in the erythema response of five individuals during various seasons. The top diagram is of a patient with cold urticaria, the remainder relate to normal subjects.

Response from symmetrical sites

In fifty subjects, the cryo-stimulation test was performed on the flexor surfaces of the forearms on both sides. Difference in the extent of erythema on the two sides was nil in twenty-three subjects, 1 mm in eleven subjects, 2 mm in thirteen subjects and more than 2 mm in three subjects. Difference in the time for which the erythema persisted was nil in six subjects, 10 s in thirty-two subjects, 20 s in eight subjects and more than 20 s in four subjects.

Thus in forty-seven out of fifty subjects, the difference in diameter of the erythema response on the two sides was not more than 2 mm, which is insignificant. Similarly, differences in the duration of erythema were also negligible.

Seasonal variations

In five individuals, including four normal subjects and one patient with cold urticaria, the cryo-stimulation test was repeated every month for 1 year. It was observed (Fig. 2) that during summer months (April–September) the response was relatively more pronounced in all individuals as compared to the response obtained during winter months (October–March). This variation was even more striking in the patient with cold urticaria.

RESPONSE

Normal subjects

This group included fifty individuals, twenty-eight males and twenty-two females, ranging in age from 11 to 52 years and not suffering from urticaria. The diameter of the erythema response was 33 mm in eleven, 34 mm in nine, 35 mm in eight, 36 mm in twelve, 37 mm in four, 38 mm in four and 39 mm in two. The duration of erythema was less than 1 min in seven, 1–2 min in twenty-six, 2–3 min in twelve, 3–4 min in four and more than 4 min in one subject (Fig. 3).

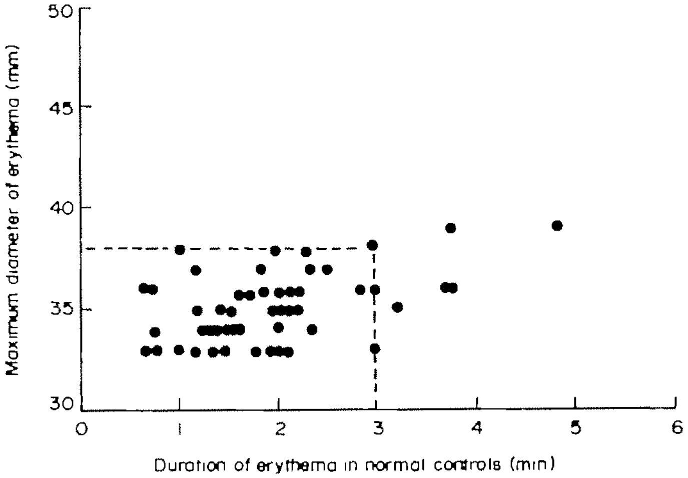

FIGURE 3. Response of fifty normal individuals to the cryo-stimulation test. The broken line represents an arbitrary limit of normal response.

If an erythema response of diameter 38 mm and duration of 3 min be accepted as an arbitrary limit of normal, only five of fifty subjects show a response outside these limits. Three of these exceed these limits only in the duration of erythema. The other two were both white British subjects. The age and the sex of the individual seemed not to affect the response in any significant manner.

Patients with cold urticaria

Attacks of cold urticaria were precipitated by exposure to cold wind, cold baths, drenching in the rain, sitting under a fan after physical exercise, and a cooled drink. Most of these patients complained of urticarial attacks during winter months only. In this study a patient was considered to have cold urticaria only if there was a definite and consistent correlation of the urticarial attacks with at least three of the above mentioned stimuli. With this criterion thirty-two patients could be designated as clinically definite cases of cold urticaria. In this group, thirty patients reported the occurrence of urticaria on exposure to cold winds, thirty-two on taking a cold bath, twenty-five on drenching in the rain, seventeen on sitting under a fan while sweating and two on taking a cooled drink. The main stimulus in all cases would seem to be low temperature, for a hot bath did not cause urticaria, and the patients whose attacks were induced by sitting under a fan developed urticaria only if they felt cooling of their skin. Some patients developed urticaria while washing their hands with cold but not with warm water. The lesions were itchy weals of variable size. severe attacks were occasionally accompanied by headache. Further details of these patients will be presented separately; all had idiopathic acquired cold urticaria. The response of these patients to the cryo-stimulation test is shown in Fig. 4. With four exceptions, all of them showed an exaggerated response. In eighteen patients both the extent and duration of erythema were increased, in nine patients only the extent of erythema was

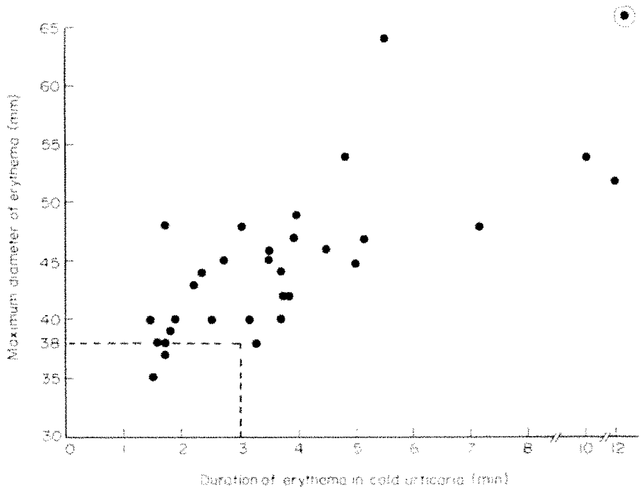

FIGURE 4. Response of thirty-two patients with cold urticaria to the cryo-stimulation test. The broken line represents an arbitrary limit of normal response. The patient indicated by ⊙ showed a response beyond the limits of this diagram and also developed a weal.

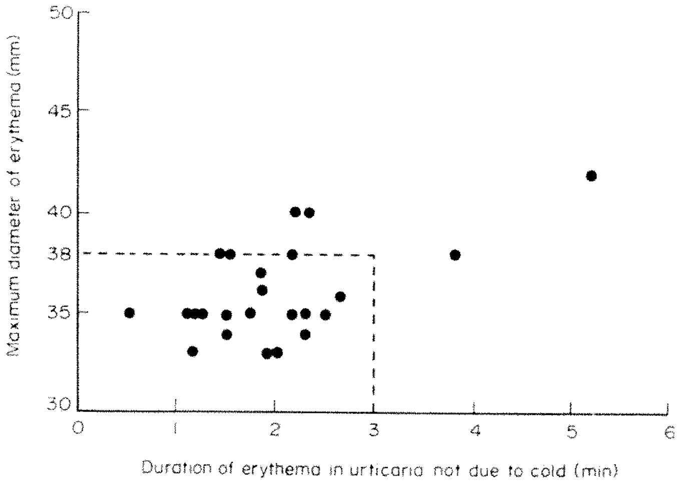

FIGURE 5. Response of twenty-four patients with urticaria of unknown cause to the cryo-stimulation test. The broken line represents an arbitrary limit of normal response.

increased, while in one patient only the duration of erythema was increased. In one patient the tested site developed a weal.

Seventeen patients in this group were tested also by the cold water immersion test by dipping one hand in cold water at 15°C for 5 min. Only one patient developed wealing on the hand. This was the same patient who had developed wealing in the cryo-stimulation test.

Patients with urticaria of unknown cause

In this group of twenty-four patients the cause of urticaria was not known. The response of twenty was within the normal range; in two only the extent of erythema was increased (40 mm) and in one only the duration of erythema was increased (3 min 50 s). One patient showed an erythema response 42 mm in diameter lasting for 5 min 10 s (Fig. 5).

DISCUSSION

This study suggests that the patients with cold urticaria react in an exaggerated manner to the cryo-stimulation test and that, in conjunction with clinical criteria, this test can be usefully employed in the diagnosis of such cases. The essential components of the stimulus in this test, the temperature of the exposure surface, the degree of pressure used for stimulation and the area of skin which is stimulated, have all been standardized. Di Palma, Reynolds & Foster (1942) have shown that the application of a 500 g weight on the skin of an individual produces a reactive hyperaemia. Application of our instrument without cold for 2 min produced only a transient erythema at the margin of the pressure area. This erythema was quite insignificant compared to the erythema obtained after the cryo-stimulation test. Nevertheless, when the weight of the instrument was increased, the erythema response to the cryo-stimulation test also increased. This indicates the need to use a standard pressure while performing the test.

In comparison to the ice cube method, where the indication of an abnormal response is the development of a weal, in the cryo-stimulation test the indicator of an abnormal response is an exaggerated erythematous reaction which can be easily quantified. This makes the cryo-stimulation test far more sensitive as compared to the other tests. By the ice cube method only one case out of thirty-two would have been confirmed as compared to twenty-eight by the cryo-stimulation test. The results of cold water immersion testing were no better. Why most of these patients with cold urticaria did not develop a weal in this test requires further investigation, but it is possible that this stimulus, although not sufficient to evoke a clinical weal in most cases, is, nevertheless, able to initiate the same set of changes as occur in clinical cold urticaria.

The incidence of false negative and false positive reactions is sufficiently low. Two of the false positive reactors were the only white British subjects included in this study, which suggests that certain races may have an exaggerated response. Subsequently, we have tested five natives of Afghanistan, all of whom showed an exaggerated response. This suggests that the upper limit of a normal response may have to be determined separately for different races before this test is adopted. Another source of false positive reactions can be the exaggerated response seen in normal individuals during summer months. This, however, cannot explain the false positive responses seen in some of our normal controls, because all these abnormal responses were obtained in winter months.

The mechanism of this test is presently under investigation, but it is considered that diseases having an increased number of mast cells in the skin are likely to give false positive reactions. The only case of cutaneous mastocytosis tested so far gave an abnormal response. A study of patients suffering from several other diseases is being undertaken to assess the true incidence of false positive reactions.

The simplest explanation for a negative test in a patient with lesions clinically suggestive of cold urticaria is a wrong diagnosis, but other factors may also have to be considered. For instance, it has been observed that if the test is repeated at the site of a previously abnormal response, the second test gives a normal response. This refractory phase is a purely local phenomenon, because the test at other sites continues to be abnormal. In three of the four patients with cold urticaria who gave a normal response there was a history of a recent attack of urticaria, and it is possible that the test site was in a refractory phase. The influence of certain drugs, particularly antihistamines and corticosteroids, will also have to be studied.

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Experimental Trichophyton infection of intact human skin

GURMOHAN SINGH

Section of Skin and Veneral Diseases, Institute of Medical Sciences, Banaras Hindu University, Varanasi-5, India

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SUMMARY

A simple new method of producing experimental *Trichophyton rubrum* infection in man is discussed. A suspension of fresh culture using a granular colony was applied under occlusion on areas of skin. Occlusion was removed after 5 days and a semi-occlusive dressing applied. It was possible to induce typical ringed or discoid lesions, which could persist for a long time if semi-occlusive dressings were continuously applied.

Different methods have been used to produce experimental infection of human skin, with varying degrees of success. Knight (1972) has recently reviewed the subject. Sloper (1955) used small fragments of pure culture or loops of suspension of culture in broth, employing *T. rubrum*, *T. mentagrophytes*, and *Epidermophyton floccosum*, and was successful in producing lesions on intact skin of the trunk and limbs. Baer (1955) produced asymptomatic infection of the feet in 50% of his subjects. Strauss & Kligman (1957) using a different method for producing experimental tinea pedis were successful in 50% of subjects at most. Desai & Bhatt (1960) described a method by which dermatophyte infection of skin abraded with sand paper, was consistently produced. Reinhardt (1971) inoculated the area with fungus spores and covered it with moistened gauze, which in turn was pressed with a teflon film to achieve occlusion and hydration.

The present paper describes a simple method for producing infection with *Trichophyton rubrum*, and its course under different experimental and natural conditions.

MATERIAL AND METHODS

Thirty adult male volunteers, free from any skin infection, were the subjects of study. Informed consent was obtained prior to study. All used a bland soap for 1 week before and during the study. They were instructed not to use any medicated toiletry during this period.

A fresh culture of *T. rubrum* (granular strain) grown on Sabouraud's medium was gently scraped from the surface and tipped into a tube containing sterile distilled water. It was thoroughly mixed for about 10 min. It was then diluted with sterile distilled water to make it only slightly hazy. Initially, very heavy suspensions or direct culture material were used, but this resulted only in a very severely inflammatory lesion, which healed very quickly.

0·5 ml of this suspension, which contained both spores and mycelial fragments, was applied to the site (pretreated with alcohol) to be infected, and occluded with 4 cm square of a non-porous, completely occlusive polythene, which was held in place with adhesive tape for 5 days. In initial experiments occlusion time was varied from 1 to 10 days, but 5 days was considered optimum. Patches were then removed; one side was left uncovered and the other covered with a semi-permeable patch of Telfa, which was removed and replaced daily.

Sites tested were; flexor aspect of forearms (five subjects), outer wall of axilla (ten subjects), medial side of upper part of thigh (ten subjects).

The character of lesions was recorded bi-weekly, followed by scrapings for KOH examination. Scrapings were cultured every week. This was continued until either the lesions completely healed, or KOH preparations became negative on two consecutive occasions with one negative culture, or the subject did not report for study, or a period of 16 weeks had elapsed.

Observations

On removal of occlusion after 5 days, lesions were similar on all the sites. Infections on forearms dried up quickly, and in four of five subjects healed completely within 1 week; the sites were negative for fungus by KOH and culture. In some subjects the site took 2½ weeks to heal and become negative. The Telfa pad covered sites healed almost as quickly as the open ones.

The results obtained on axillae and thighs were essentially similar and are analysed together. Subjects were followed for varying periods of time; 7–10 weeks in five subjects, 13–16 weeks in thirteen subjects, and two subjects were followed for only 3 weeks.

When the occlusion was removed the area was observed to be macerated with papulo-vesicular lesions, which became scaly in a day or two. In a majority of sites the lesions were more pronounced at the periphery, probably because the suspension of fungus usually settled at the borders of the polythene patch, due to capillary action.

In two subjects lesions were not diffuse and did not involve the whole area under the patch, but instead three to four isolated annular lesions developed on Telfa occluded sites, resembling classical ringworm (Fig. 1). On the opposite site the lesions healed quickly, within 5 days in both the subjects.

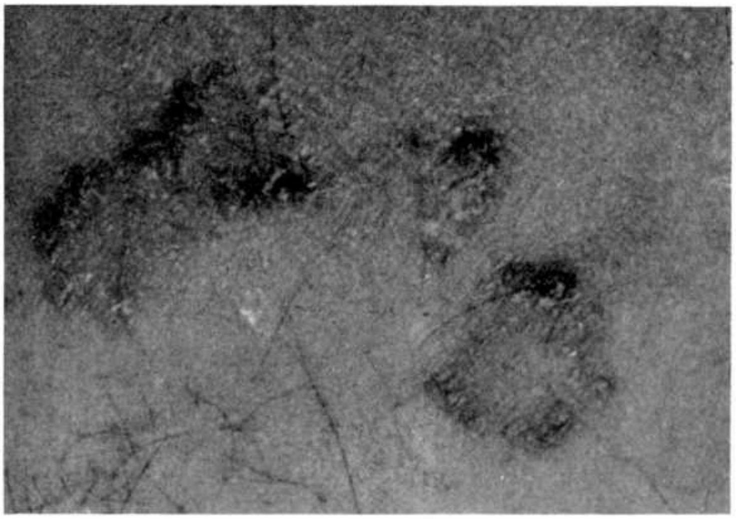

FIGURE 1. Experimental *T. rubrum* infection after occlusion.

In two subjects reaction was markedly inflammatory and the lesions subsided with complete healing within 1 week on both sides. After this period the skin became practically normal and was negative for fungus.

In the majority of subjects (sixteen out of twenty) occluded with Telfa, lesions remained stationary for a period ranging from 5 to 10 days in different subjects. After this latency there was activity at some sites, as shown by a papulo-vesicular eruption assuming a circinate appearance (Fig. 2). Such a characteristic developed in nine out of sixteen lesions which were continually occluded with Telfa patches. The uncovered lesions showed such a picture on three sites only, and the time between the removal of occlusions and the development of circinate lesions was also higher, being 9–10 days.

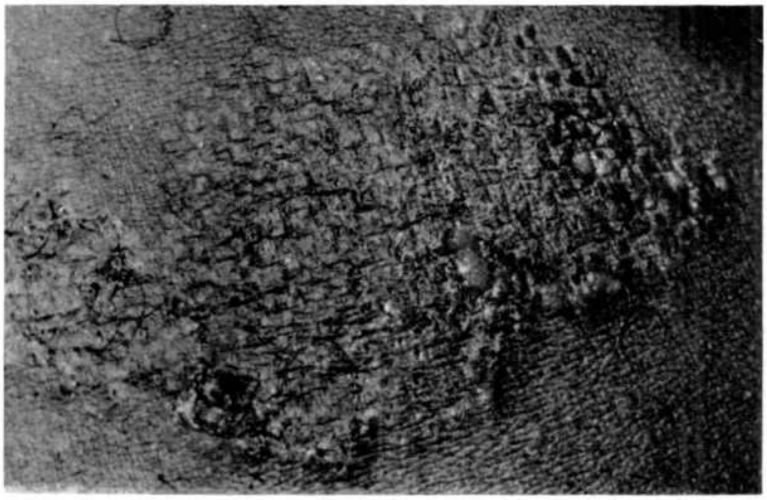

FIGURE 2. Experimental *T. rubrum* infection.

All other sites, i.e. four Telfa covered and thirteen uncovered sites, continued to show scaliness, but the thickness of patches gradually diminished. The colour also changed from erythematous to hyperpigmented. The fungus was demonstrated till the end of follow-up, a maximum period of 16 weeks. After this period the patients were treated with a topical antifungal agent until they became negative for fungus both by KOH and culture.

Two subjects who had become negative before discharge met the author on the road-side and complained of itching on and near test sites, which on examination showed typical erythemato-squamous, annular, and circinate lesions.

DISCUSSION

There are few reports where dermatomycoses have been consistently produced in human subjects under experimental conditions. Initially, procedures used included scarifying the skin; intradermal injections of spore suspensions (Strauss & Kligman, 1957); abrasion with sand paper (Desai & Bhat, 1960) and intradermal injection followed by scarification (Held & Friedman, 1962); occlusion of skin by moist gauze and sealing with teflon film. Sloper (1955) successfully produced infection without using the above techniques in a small sample of volunteers in Malaya, where the warm and humid sub-tropical climate produced moistness of skin.

The technique of occlusion with polythene results in a tropical environment on the occluded skin.

The skin becomes macerated and is said to be more susceptible to fungus infection (Ilowite, 1967). Areas were occluded for 5 days only, as this was observed to be enough for the fungus to produce lesions. It may be that the incubation period under optimal conditions is less than 5 days. Sloper (1955) observed the incubation period with *T. rubrum* to be 3 days. According to Reinhardt (1971), occlusion is necessary for at least 3 days in order to produce an established infection. Complete occlusion for a longer period resulted in maceration and inflammation becoming marked, and in self healing within 1 week. Similarly, use of a larger inoculum produced inflammatory lesions which healed quickly. Severe inflammation may mobilize local defence mechanisms or the exuded serum may act as an antifungal agent. Increase in turnover rate of epidermal cells resulting in shedding of fungal elements has also been proposed (Knight, 1972). At a later stage partial occlusion produced some local heat and humidity, with the result that lesions progressed more at these sites than in controls. In initial experiments many different semi-occlusive dressings such as Blenderm, cotton gauze, and Telfa were used; the latter was found to give the best results.

Before inoculation, areas were treated with ethanol. This was to avoid any chance of overgrowth of *Staphylococcus aureus* with production of experimental staphyloderma (Singh, Marples & Kligman, 1971).

Before developing this method the author had used one to four infected hair cuttings. *T. rubrum* grown on Sabouraud agar was scraped and ground with sterile distilled water. Hair cuttings were then soaked in this suspension, which was spread on nutrient agar plates. Fungus grew on the hair, which was then used for inoculation. The lesions produced were small; limited to the immediate vicinity of hair. The results were not very consistent and so this method was abandoned.

Earlier experimenters have given different explanations for their failure or success, including the significance of use of spores or filaments. Desai & Bhat (1960) attributed their success to the use of hyphal inoculation and not spore suspension, as used by Strauss & Kligman (1957). The author used a suspension which had chiefly spores and few hyphal elements, and was successful. It is difficult to say definitely from these experiments whether or not hyphae are essential, but the author believes that spores would germinate under optimal conditions of heat and moisture and produce filaments.

Abrasion similarly does not appear to be an essential prerequisite. During natural infection abrasion presumably does not play any role. The success obtained by abrasion or scarification by early workers may be attributed to some exudation at the area due to above procedures, which resulted in local hydration and so increased susceptibility and higher number of 'takes'. The same goal was achieved by simple occlusion with a non-toxic, completely occlusive polythene patch.

This is a simple method for producing experimental *T. rubrum* infection on intact human skin. Its greatest advantage is its practicability.

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Asymmetry of cellular response as revealed by the skin window technique

STUART A. GARRIE AND PHELPS CRUMP

Dermatology Service, Brooke Army Medical Center, Fort Sam Houston, Texas 78234, U.S.A.

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SUMMARY

Utilizing the skin window technique, symmetry of cellular response was examined by comparing cellular exudates from symmetrical areas of opposite forearms in eleven subjects. Testing the hypothesis that there would be no significant difference between symmetrical sites subjected to the same stimulus at the same time, the percentages of cell types in the cellular exudate were calculated for 126 paired comparisons. A significant difference in the cell types arriving at the skin window sites occurred in seventy-six out of the 126 paired comparisons (Chi square analysis, $P \leqslant 0{\cdot}01$). This lack of a symmetrical response could be due to an inherent variability of the cellular response or to an inability to manipulate the two sites in exactly the same manner.

Although significant differences were found between the above individual matched pairs (Chi square analysis), the '*t*' test analysis of the pairs grouped by hour revealed no significant difference in mean percentages of cell types between left and right arms at fourteen time intervals. Thus, previous skin window investigations comparing mean percentages of cell types were found to be valid, even though symmetry of cellular response in individual subjects is much less than expected.

The skin window technique is a method of recording the cellular events occurring at the skin's surface at timed intervals following an initial stimulus. It has been used to study the cell types involved in various inflammatory and neoplastic states (Ascheim *et al.*, 1966; Eidinger, Wilkinson & Rose, 1964; Fowler & Lowell, 1966; Garrie & Wolf-Jurgensen, 1971; Hautklin, 1968; Vesper, Winkelman & Hargraves, 1971; Wolf-Jurgensen, 1962, 1967; Wolf-Jurgensen, Henningsen & Zachariae, 1969; Wolf-Jurgensen & Zachariae, 1965).

To determine the cell types involved in these states, the percentages of polymorphonuclear cells, mononuclear cells, eosinophils, and basophils are calculated from skin window coverslips. These percentages are then compared to those derived from a control coverslip. The control coverslip is usually placed on a symmetrical site of normal skin on the opposite arm.

This study was initiated to determine if there is symmetry of cellular response when symmetrical sites on the forearm skin are manipulated in the same manner. In addition, this investigation checks an assumption of the previously quoted studies: that is, if symmetrical sites in several subjects are

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Correspondence to: Stuart A. Garrie, M.D., 515 Exeter Road, B6, San Antonio, Texas 78209, U.S.A. Reprint requests to: Dermatology Service, Brooke Army Medical Center, Fort Sam Houston, Texas 78234, U.S.A.

exposed to the same stimulus, no significant difference in mean cellular response will occur between opposite sites.

METHOD

Eleven subjects were studied. Six of the subjects had dermatoses; two with grade I acne, one with mild atopic dermatitis in remission, one with dyshidrosis of the hands and feet in remission, one with mild seborrhoeic dermatitis, and one with an undiagnosed minimal eczematous eruption of the feet. The other five subjects had no history of skin disease.

Each subject had symmetrical areas on the volar surface of his left and right forearm cleansed with ethanol then a 1 cm square area of skin was abraded with a scalpel until fine bleeding points were seen. A drop of physiological saline was then applied to both sites and covered with a cardboard-backed sterile glass coverslip. The coverslips were changed at 3, 6, 9, 12, 15, 21, 24, 27, 30, 33, 36, 39, 45 and 48 h, air dried, and Wright stained. The cells were counted in a fixed manner (Garrie & Wolf-Jurgensen, 1972) and the percentage of each cell type calculated.

The two forearms of each subject were then compared as matched pairs. Eosinophil and basophil percentages were less than 1% of the cells present at most time intervals, so the percentage of mononuclear cells was usually 100% minus percentage polymorphonuclear cells for each coverslip. Three analyses were performed.

(1) The proportions of cells of each type were compared between the left and right by using a Chi square 2 × 2 table analysis on each pair. An example of the analysis is given for subject D.C. at the ninth hour (Table 1).

TABLE 1. Chi square analysis of a single pair

	Arm		
Cell type	Left	Right	Total
Polymorph	348*	103	451
Mononuclear	128	90	218
Total	476	193	669

$\chi^2 = 24.261$, $P < 0.0001$
* Number of cells.

(2) A paired Student's 't' test and Wilcoxon signed rank test were used to analyse the subjects' percentages grouped at each hour. Only the 't' test results will be reported, as the Wilcoxin signed rank test gives similar significance levels. The 't' test was used to recognize any significant difference between the mean percentages of cell types on left versus right arm.

(3) An analysis of variance was applied to the data using a mixed model with subjects random and other variables fixed. This analysis was done to ascertain the interaction of subjects, time and arms (left or right) at five time intervals. Because of accidental breakage of coverslips, a full eleven subjects' coverslips were present only at 6, 15, 30, 33, and 45 hr.

RESULTS

(1) The Chi square analysis revealed that out of 126 paired left–right arm comparisons, seventy-six

TABLE 2. Analysis of variance for polymorphonuclear cell type percentages

Source	DF	Mean square	F	P
Subjects	10	2161·0^{a}	a/g = 16·86	0·001
Time	4	4437·5^{b}	b/c = 9·21	0·001
Subjects × time	40	481·7^{c}	c/g = 3·76	0·001
Arms	1	156·2^{d}	d/e = 0·25	NS*
Subjects × arms	10	627·7^{e}	e/g = 4·90	0·001
Time × arms	4	23·6^{f}	f/g = 0·18	NS
Subjects × time × arm	40	128·2^{g}		
Total	109			

*NS, $P > 0{\cdot}1$.

pairs had significant differences in percentages of cell types ($P \leqslant 0{\cdot}01$); seventy-six out of 126 is a high incidence of non-agreement between symmetrical areas of the left and right arms.

(2) The paired '*t*' test was applied to the percentages of polymorphonuclear and mononuclear cell types of the subjects grouped at each hour. Comparing left to right, there was no significant difference in the means of the cell types, except at the twenty-first hour ($P = 0{\cdot}02$ for both cell types).

(3) Table 2 shows the results of the analysis of variance for the polymorphonuclear cell type. The analysis of variance of the mononuclear cell type showed identical significance levels (*P*) to Table 2.

DISCUSSION

The Chi square results showed that there can be a significant difference in the skin window record of the cellular response when symmetrical sites are investigated in the same way. It is difficult to accept the possibility that the cellular response differed between symmetrical areas of the body treated in the same fashion; however, this has been noted with respect to sodium concentrations in sweat samples taken from two symmetrical areas on the back (Gibinski *et al.*, 1971). A more likely possibility remains; the supposedly matched pairs are not matched: (1) the skin window sites may not be placed on precisely the same symmetrical sites, (2) the depths of abrasion could be different, (3) the pressure from the tape over the coverslips may vary, etc.

One might ask why the Chi square analysis of individual pairs revealed a very significant difference in the cellular responses, yet the '*t*' test analysis did not? The answer is that the tests measure two different relationships. The Chi square analysed differences between each pair of coverslips as a single unit; the '*t*' test compared groups of paired coverslips (all the subjects at each hour), i.e. a relationship between two means (left and right). Although there were marked differences between many of the individual pairs, sometimes the differences occurred with the right predominating and sometimes the left; so when the right and left were totalled and averaged, they balanced out to show no significant difference. Table 3 shows how this occurred at the end of the fifteenth hour. The means for left and right were not significantly different; but one can see that there were marked differences present in each individual pair. The lack of a significant difference ($P > 0{\cdot}10$) between left and right arms over five time intervals can be explained on the same basis (Table 2, arms).

This absence of a significant difference in mean percentages of cell types between left and right arms helps validate previous skin window studies.

TABLE 3. Fifteenth hour comparison of left and right arms

Left %P*	Right %P
81·6	67·5
29·2	34·5
85·3	71·1
50·7	41 4
71·6	41·8
27·1	47·6
62·4	38·7
5·0	14·3
1·0	3·8
25·1	32·1
46·1	43·9
N=11†	
Mean 44·1	39·7
P=0·36‡	

*Polymorphonuclear cell type percentages, †number of subjects, ‡probability of '*t*' test using a two-sided hypothesis.

Skin window studies may be divided into broad areas: cellular response in diseased versus non-diseased skin, or cellular response to an antigen versus a no-antigen control.

In the first class of investigations falls Ascheim's study of skin windows put on involved and uninvolved psoriatic skin (Ascheim *et al.*, 1966). Another is Vesper's study of subjects with mycosis fungoides, where diseased skin is compared to the '... contralateral normal skin...' (Vesper *et al.*, 1971). The psoriasis study revealed no mean differences in percentages of cell types at various hours; on the other hand, in the early hours of inflammation the mycosis fungoides plaques were noted to have higher median percentages of mononuclear cells than uninvolved skin.

Several studies with antigens have been performed (Eidinger *et al.*, 1964; Feinberg, Feinberg & Fumiko, 1967; Hautklin, 1968; Wolf-Jurgensen, 1962, 1967; Wolf-Jurgensen *et al.*, 1969; Wolf-Jurgensen & Zachariae, 1965). They all postulate that the percentage differences in cell types between control and antigen treated sites is due to the antigen's influence.

These two classes of investigations were usually performed on several subjects grouped by hour. Thus, our '*t*' and rank test results apply. If our study had shown a significant difference between means, when both arms were treated with saline, it would have called into question the conclusions of the aforementioned studies, e.g. that the difference between means was really due to the antigen or disease state.

Previous investigators (e.g. Ascheim *et al.*, 1966; Wolf-Jurgensen, 1962) also have noted the change from polymorphonuclear cell predominance in the early hours of inflammation to mononuclear cell predominance in the late hours (leukocytic cycle); this was verified in our study (Table 2, time). Although there was a change in cellular response with time, the amount significantly varied from subject-to-subject (Table 2, subject × time).

Two limitations are suggested by our study. Several subjects (seven out of eleven) were found by chance to have a particular cell type predominating on the same arm at most time intervals: e.g. subject D.C. had higher percentages of polymorphonuclear cells on his left compared to his right arm at thirteen out of fourteen time intervals. Thus, in small samples, the predominance of arms could lead to a presumed antigen effect on a particular cell type if the antigen got on only 'predominant' arms. One method of overcoming this is to randomize antigen placement; previous studies have not mentioned such a randomization of antigen placement.

Our second recommendation is that rather large numbers of subjects should be included in a skin window study. The exact number cannot be specified, for it depends on the degree of antigen effect. In the analysis of variance, significant variation between subjects was found (Table 2, subjects). Also, no consistent difference between left and right arms occurred between subjects (Table 2, subject × arms); perhaps this interaction is due to influences mentioned under the discussion of Chi square results. These findings lead to the suggestion that numerous subjects should be examined to avoid type II errors, that is, missing a significant difference when one is present.

Further investigations suggested by this study are comparisons of cell types when both skin window sites are treated the same way on the same arm instead of on opposite arms symmetrically. Also, an antigen which elicits eosinophils or basophils should be applied to both symmetrical sites on allergic and non-allergic subjects. A further study on single subject variation could be done by applying skin window sequences on the same subject at different times (e.g. at monthly intervals) to see if left or right arm predominance is a constant feature in a single subject.

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The uninvolved psoriatic epidermis

A. JARRETT

Dermatology Department, University College Hospital Medical School, University Street, London, W.C.1

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SUMMARY

Normal control and apparently unaffected skin from psoriatics have been compared quantitatively for their acid phosphatase activity. The unaffected skin from psoriatics shows a more rapid reaction, which may be related to a lysosomal defect.

No marked differences were detected in the dehydrogenase reactions in these two tissues. However, in frankly involved psoriatic skin it has previously been shown that there is a marked increase in glucose-6-phosphate dehydrogenase activity in the transitional zone. In the present report an increase in glycerophosphate dehydrogenase activity has been demonstrated in the lower portion of psoriatic epidermis.

Over the years, it has become increasingly evident that the unaffected skin of psoriatics is abnormal. The first suggestion that the apparently normal skin of the psoriatic might differ from normal was the work of Wedell *et al.* (1965). They showed that there is a greatly increased number of nerves and Schwann cells in unaffected psoriatic skin, and suggested that the cellular infiltrate of the psoriatic plaque is not exclusively composed of inflammatory cells as is usually thought, but that Schwann cells are also present in all stages of development, degeneration, and decay. Differences between the reaction of apparently normal psoriatic skin and of normal control skin to the local application of vitamin A have also been demonstrated (Reid & Jarrett, 1967). The unaffected psoriatic epidermis was much more thickened than the control epidermis, and there was a much more intense reaction for hydrolytic enzymes of the lysosomal type. This indicated that the lysosomes in normal psoriatic epidermis are more greatly affected by lysosomal labilizers than those of truly normal epidermis.

It should be mentioned that the clinically normal psoriatic epidermis shows no obvious abnormality when examined by routine haematoxylin and eosin staining. It is only with special investigations, or when the skin is stimulated into proliferative activity, that differences become manifest. It appeared that the uninvolved psoriatic epidermis was thicker than normal control epidermis, but when this was tested statistically it failed to reach biologically significant levels (Reid & Jarrett, 1967).

MATERIAL AND METHODS

Uninvolved skin from patients with psoriasis was biopsied, and these specimens were matched for age, sex, and site, with skin from normal controls who had never had psoriasis, nor any family history of the disease.* The acid phosphatase activity of these specimens was quantified in the mid-epidermal region (mid-way between the granular layer and the basal cells: see Fig. 1) by continuously monitoring the reaction by microphotospectrometry, using the method already described by Jarrett & Please (1970). Other biopsies, from normal control and uninvolved psoriatic skin, and from psoriatic plaques were examined for glucose-6-phosphate dehydrogenase, glycerophosphate dehydrogenase, and glyceraldehyde-3-phosphate dehydrogenase activities.

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* Biopsy material for this part of the investigation was kindly supplied by Dr T.W.E.Robinson of University College Hospital.

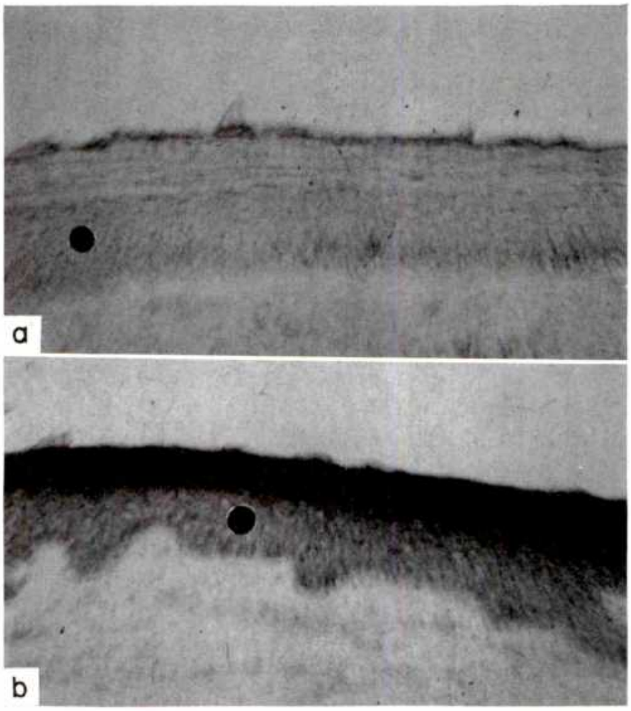

FIGURE 1. (a) Normal control epidermis: reaction for acid phosphatase, 24 min dye coupling alpha-naphthol phosphate and fast red TR salt. (b) Apparently normal skin from psoriatic: reaction for acid phosphatase, 24 min reaction in same substrate as normal control. Note the much greater reaction in the apparently normal epidermis. The black areas show the approximate regions from which the quantification was made, being mid-way between the granular layer and the basal layer.

All the biopsy material was frozen, unfixed, onto crytostat chucks and sectioned at 8 μm. Skin examined for acid phosphatase activity was incubated in a medium containing alpha-naphthol phosphate and fast red salt TR (alpha-naphthol phosphate (Sigma) 4 mg, 0·15 M veronal-acetate buffer pH 5·4 5 ml, distilled water 15 ml, fast red salt RT (Gurr) 5 mg). The substrate was made up immediately before each experiment and filtered before use. The sections were immersed in the incubating medium in a heated chamber and the formation of the insoluble red azo dye monitored.

The material examined for dehydrogenase activity was incubated in the following medium at 37°C and examined after 30 min, 2 and 18 h. The sections were then fixed in 5% formalin for 5 min, washed, and finally mounted in water mounting medium (Gurrs) (nicotinamide adenine dinucleotide phosphate (NADP) (Sigma) 5 mg, DL-glyceraldehyde-3-phosphate (or DL-alpha glycerophosphate) 15 mg, nitro blue tetrazolium (Nitro BT) (Sigma) 5 mg, magnesium chloride 0·015 M

0·2 ml, 0·2 M veronal-acetate buffer pH 7·4 5·0 ml, distilled water 4·8 ml). Both nicotinamide adenine dinucleotide phosphate (NADP) and nicotinamide adenine dinucleotide (NAD) were used as co-enzymes in the dehydrogenase systems.

RESULTS

Acid phosphatase

Linear regression coefficients (b_{24}) for a period of 24 min were calculated from the tracing obtained from the labograph recorder connected to the galvanometer. For reasons already published, it was thought adequate to use these simple linear coefficients rather than corrected readings, because they were to be used for comparison and not as absolute values (Jarrett & Please 1970). The values given in Table 1 are arithmetical averages of readings taken from two, and in some cases three, sections from the respective biopsies.

TABLE 1. Regression coefficients

'Normal skin' from psoriatic mid-epidermis ($b_{24} \times 10^3$)	Normal control skin matched for age, sex and site, mid-epidermis ($b_{24} \times 10^3$)
−4·74 (calf)	−3·03 (calf)
−5·26 (shin)	−3·04 (shin)
−13·90 (thigh)	−3·76 (thigh)
−7·00 (elbow)	−4·52 (elbow)
−5·19 (forearm)	−1·95 (forearm)
−4·21 (back)	−1·50 (back)
−3·67 (shoulder)	−1·97 (shoulder)

In all cases tested it was found that the mid-epidermis of apparently normal psoriatic skin showed a greater enzyme activity than the corresponding region of normal control epidermis (Fig. 1 and Table 1). The difference between these two sets of regression coefficients was statistically significant: $t = 2{\cdot}299$; $P > 0{\cdot}05$ for 12 degrees of freedom.

The acid phosphatase reaction in frankly involved epidermis from psoriatic plaques gave a very uneven reaction, with regions of very high activity and others of relatively low activity. The highly active sites appeared to be related to the presence of migratory cells within the psoriatic epidermis, and it was probably their content of acid hydrolases that augmented the local reaction. For this reason it was not possible to obtain meaningful quantifications of the acid phosphatase reaction in psoriatic epidermis, and therefore estimations were restricted to normal and uninvolved psoriatic epidermis (Table 2).

Dehydrogenases

In relation to the pentose phosphate shunt, it has been reported that frankly involved epidermis shows a strong reaction for glucose-6-phosphate dehydrogenase in the transitional zone of the epidermis (Rassner, 1965, 1966; Jarrett, 1968, 1971). The presence of this enzyme has also been demonstrated in embryonic skin (Hashimoto & Lever, 1966), and in primate skin by Im & Adachi (1966).

Virtually no activity was detected in normal epidermis and only very slight activity in uninvolved skin from psoriatic patients; this was in marked contrast to the psoriatic plaque.

A weak glycerophosphate dehydrogenase reaction was detected in the lower epidermis of normal and uninvolved psoriatic epidermis. The frankly involved psoriatic epidermis, however, showed a strongly positive reaction in the lower epidermis (Fig. 2).

Negative results were obtained for glyceraldehyde-3-phosphate dehydrogenases in all the material examined.

TABLE 2. To illustrate the great variation of hydrolytic activity within frankly psoriatic epidermis. These gross variations preclude the quantification of acid hydrolases in psoriatic epidermis

	Activity from different regions of psoriatic epidermis (b_{24} regression coefficients × 10^3)		
	Area 1	Area 2	Area 3
Case 1	−1·06	−7·82	—
Case 2	−3·46	−3·50	12·5

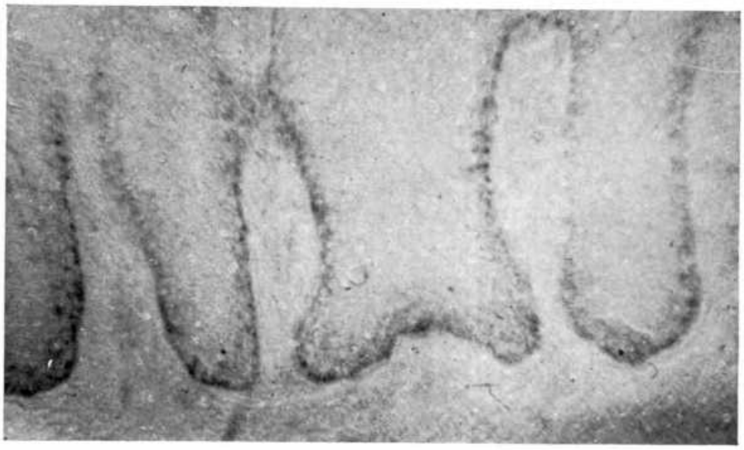

FIGURE 2. Psoriatic epidermis: reaction for DL-alpha-glycerphosphate dehydrogenase. Note the reaction in the lower layer of the acanthotic epidermis.

None of the reactions, except for the glucose-6-phosphate dehydrogenase in psoriatic epidermis, was sufficiently strong or rapid to enable worthwhile quantification over a period of 24 min. The quantitative results obtained with this material have already been reported (Jarrett, 1971).

The results with these enzymes using co-enzymes NAD and NADP are given in Table 3.

TABLE 3. Dehydrogenases in human epidermis

	Normal control	Uninvolved psoriatic epidermis	Psoriatic plaque
Glucose-6-phosphate dehydrogenase.	Negative→trace	Trace→slight	Strongly positive
Co-enzyme NADP	Transitional zone	Transitional zone	Transitional zone
Glucose-6-phosphate dehydrogenase.	Negative	Trace	Positive
Co-enzyme NAD		Transitional zone	Transitional zone
Glyceraldehyde-3-phosphate dehydrogenase.	Negative	Negative	Negative
Co-enzyme NADP or NAD	All regions	All regions	All regions
Glycerophosphate dehydrogenase.	Very slight activity	Slight activity	Positive reaction
Co-enzyme NAD	Lower epidermis	Lower epidermis	Lower epidermis

DISCUSSION

The increase in acid phosphatase activity in uninvolved skin from a psoriatic could result either from an increase in the number of lysosomes in the epidermis or from an abnormal instability of their membranes. An abnormality of the membrane would facilitate penetration of the substrate into, or diffusion of the enzymes out of, the lysosomes. Either of these would give a more rapidly developing reaction than a tissue in which the lysosomal membranes were more stable. When normal control and uninvolved psoriatic epidermis were incubated for more prolonged periods of up to 12 h, the end products showed similar intensities, and this indicated a defect in the membrane rather than an absolute increase in the number of lysosomes.

Cytochemical electron microscope techniques based on the Gomori reaction for acid phosphatase failed to help in this matter. Electron microscopy does not lend itself readily to accurate quantification and, as already mentioned, the differences between normal skin and uninvolved psoriatic skin become obscured after more prolonged incubation times. It is hoped that with more advanced cytochemical techniques some of these difficulties will be overcome, because it is only by electron microscopy that we shall be able to establish definitely whether or not there is an absolute increase in the number of lysosomes.

These results confirm those obtained earlier with normal and uninvolved skin after the application of vitamin A (Reid & Jarrett, 1967). It was shown that after the topical application of this vitamin very much more acid phosphatase activity was induced in uninvolved epidermis of the psoriatic than in epidermis of normal controls. The degree of epidermal hyperplasia was also very much greater. As vitamin A is a lysosomal labilizer, these observed effects could be interpreted as evidence for an abnormal instability of the lysosomal membrane.

The possible explanation for the greatly increased glucose-6-phosphate activity in frankly psoriatic epidermis has already been considered (Jarrett, 1971). It would appear unlikely that the breakdown of glucose by the pentose phosphate shunt continues by way of glyceraldehyde-3-phosphate dehydrogenase, as no activity of this enzyme could be detected, even in frankly involved psoriatic epidermis.

Glyceraldehyde-3-phosphate is produced by the pentose shunt when ribose-5-phosphate is further degraded. Although this material could be utilized for the formation of ribonucleic acid, it is unlikely that it would occur at this high epidermal level.

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A comparison between DNA synthesis and mitosis in uninvolved and involved psoriatic epidermis and normal epidermis

PETER GOODWIN, SUSAN HAMILTON AND LIONEL FRY

Department of Dermatology, St Mary's Hospital, London, W.2

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SUMMARY

Biopsies have been taken from the involved and uninvolved skin of eleven patients with psoriasis and the normal skin of six patients without psoriasis, 1 h after *in vivo* injection of 10 μCi of ^{3}H-thymidine. Mitotic counts and counts of labelled cells (synthesizing DNA) after autoradiography have been carried out. The ratio of mitoses to labelled cells was 1 : 25 in clinical psoriasis, 1 : 190 in uninvolved psoriatic epidermis and 1 : 450 in normal epidermis. The number of labelled cells in clinical psoriasis was approximately 12 times greater than normal epidermis. However, the number of labelled cells in uninvolved psoriatic epidermis was approximately twice that of normal. These results imply an alteration in the components of the cell cycle in both involved and uninvolved psoriatic epidermis.

One of the characteristic features of psoriatic epidermis is the increase in the number of cells seen to be in mitosis. Compared to normal epidermis, there is no increase in the number of cells in mitosis in the uninvolved psoriatic epidermis (Wohlrab & Gruneberg, 1966). However, recent *in vitro* studies have shown that the number of cells synthesising DNA is increased in the uninvolved psoriatic epidermis compared to normal epidermis (Hell & Hodgson, 1966; Braun-Falco, Christophers & Kurban, 1967; Kaidby & Kurban, 1971), although *in vivo* studies by Weinstein & Frost (1968) found the number of cells synthesizing DNA to be the same as in normal skin. Thus, it is possible that the increase found in the *in vitro* studies was due to the actual biopsy or to environmental factors and that there is no real increase in the proportion of cells synthesizing DNA in uninvolved psoriatic epidermis compared to normal epidermis.

The number of cells in mitosis in psoriatic epidermis has been found to be in excess of 50 times that in normal epidermis (Fry & McMinn, 1968, 1970). However, the increase in DNA synthesis in psoriatic epidermis has been reported to be only approximately 5 times that of normal skin (Weinstein & Frost 1968).

Thus, there is a suggestion that the relationship between DNA synthesis and mitosis is not a 'fixed one', and implies possible alterations of these components of the cell cycle both in the uninvolved and involved psoriatic epidermis compared to normal. We have, therefore, undertaken an *in vivo* study to compare the relationship of DNA synthesis to mitosis in the epidermis in these three situations.

TABLE 1. Comparison between average totals of DNA synthesizing cells and cells undergoing mitosis in the epidermis (counts per 100 high power fields)

	Involved psoriasis	Uninvolved psoriasis	Normal
Mitotic count	75 (± SE 8·5)	1·9 (± SE 0·5)	0·33
Labelled cell count	1745 (± SE 75)	362 (± SE 32)	150 (± SE 19)
Ratio mitotic count : labelled cell count	1 : 25	1 : 191	1 : 450

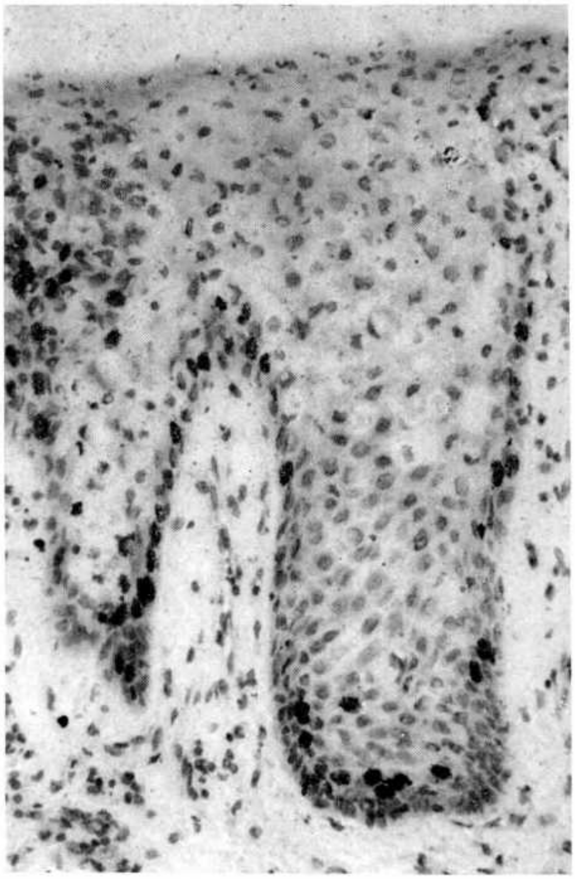

FIGURE 1. Autoradiograph of involved psoriatic epidermis showing labelling pattern 1 h after *in viva* ^{3}H-thymidine injection. Stained with Ehrlich's haematoxylin (× 240).

PATIENTS AND METHODS

Eleven patients with psoriasis and six patients with other skin disorders were studied. All the latter six patients were having wide excisions of skin lesions (two had basal cell carcinomata and four had benign papillomata). The skin studied was at least 1 cm from the clinical lesion. All patients were over the age of 50, and the nature of the investigation was explained to them. In the patients with psoriasis the biopsies were taken from the edge of a plaque and from uninvolved skin at least 5 cm from a plaque. 1 h prior to biopsy 10 μCi of ^{3}H-thymidine was injected into the skin. The biopsy was carried out under local anaesthesia, using 2% lignocaine, with a 4 mm punch. The specimens were fixed in formol saline, embedded in wax and sections 5 μm thick cut. Mitotic counts were obtained from sections stained with haematoxylin and eosin. Labelled cells were counted from autoradiographs prepared by coating sections with Kodak AR 10 stripping film and incubating for 3 weeks at 4°C. In all specimens 100 high power fields (×400) were counted, as described by Fry & McMinn (1966).

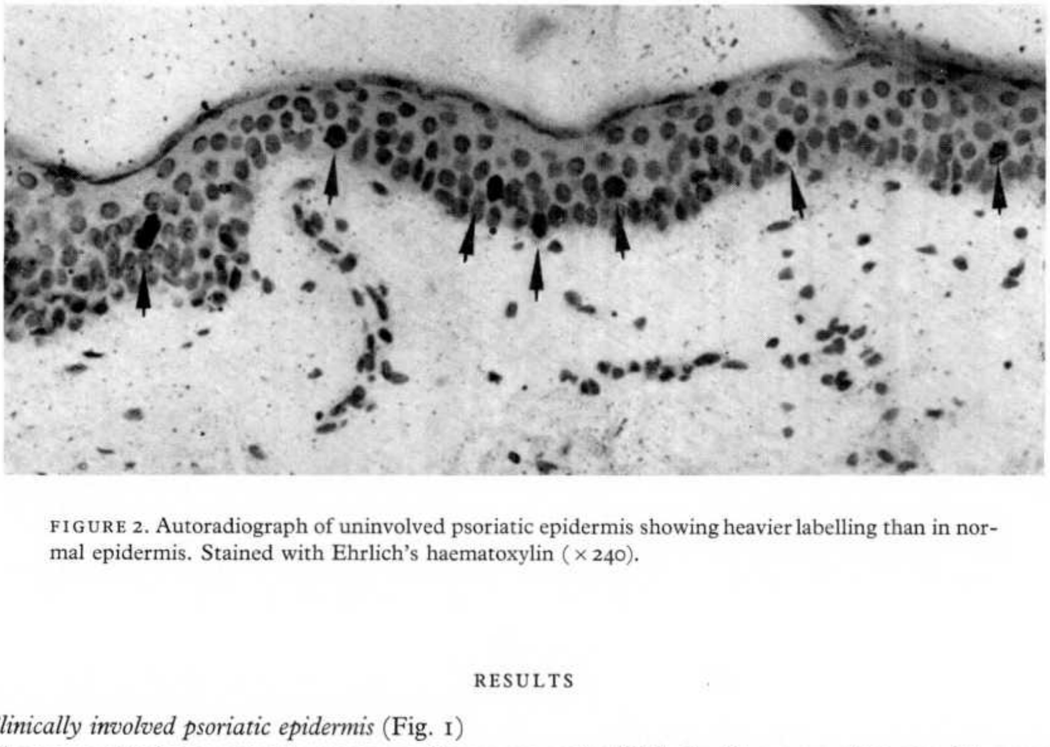

FIGURE 2. Autoradiograph of uninvolved psoriatic epidermis showing heavier labelling than in normal epidermis. Stained with Ehrlich's haematoxylin (×240).

RESULTS

Clinically involved psoriatic epidermis (Fig. 1)
The mean mitotic count in the eleven specimens was 75 (± SE 8·5) with a range of 50–139. The mean number of cells labelled with ^{3}H-thymidine was 1745 (± SE 75) with a range of 1522–2249. The mean ratio of mitoses to labelled cells was 1 : 25.

Uninvolved epidermis from patients with psoriasis (Fig. 2)
The mean mitotic count was 1·9 (± SE 0·5) with a range of 0–5. In the specimens from two of the eleven patients no mitotic figures were seen. The mean number of labelled cells was 362 (± SE 32) with a range of 210–620. The mean ratio of mitoses to labelled cells was 1 : 191.

Normal epidermis (Fig. 3)

The mean mitotic count was 0·33. However, in four of the six specimens no mitosis was seen. The mean number of labelled cells was 150 (± SE 19) with a range of 87–200. This gives a ratio of mitoses to labelled cells of 1 : 450.

Thus, in the involved psoriatic epidermis compared to the uninvolved, the number of mitotic figures is increased approximately 40 times, while cells labelled with ^{3}H-thymidine were only increased approximately 5 times. Compared to normal skin there is a definite increase in the number of labelled cells in the uninvolved psoriatic epidermis of the order of 2·4 times. However, there also appear to be more mitotic figures in the uninvolved psoriatic epidermis, but even so the ratio of mitotic figures to labelled cells seen is nearly twice as much as in the normal skin.

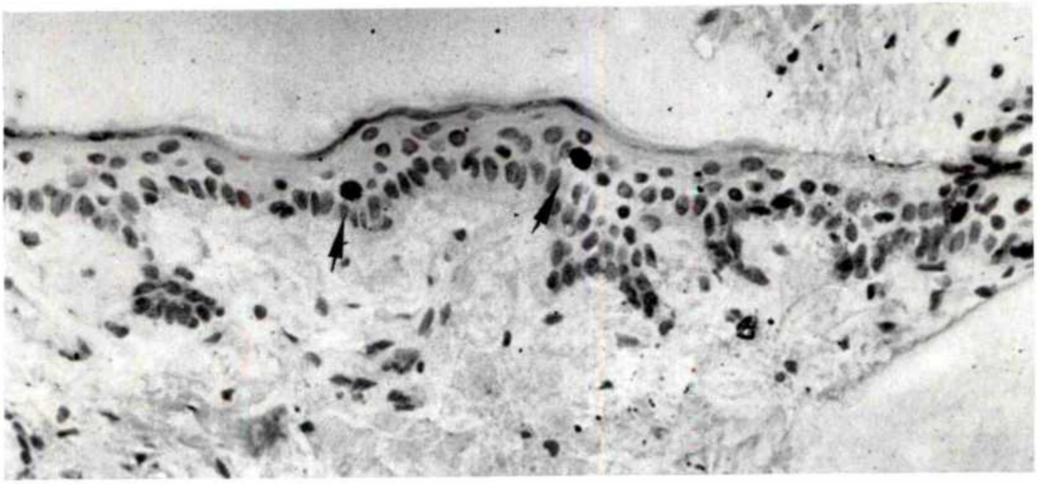

FIGURE 3. Autoradiograph of normal epidermis. Stained with Ehrlich's haematoxylin (× 240).

DISCUSSION

These results show two significant points. First, that proportionately there is considerably greater increase in the number of mitotic figures in clinical psoriasis than in cells labelled with ^{3}H-thymidine in uninvolved psoriatic skin. Second, that *in vivo* there are more cells labelled with ^{3}H-thymidine in uninvolved psoriatic epidermis than in to normal epidermis.

In order to explain the disproportionate increase in mitosis in involved psoriatic epidermis, it is necessary to refer to the concept of the cell cycle. In a balanced cell system, such as the epidermis, it is normally assumed that germinal cells undergoing DNA synthesis proceed to mitosis. The ratio of the number of cells in DNA synthesis (S phase) to the number of cells undergoing mitosis should be equivalent to the ratio of the duration of these two phases of the cell cycle, i.e:

$$\frac{\text{Number of cells in S phase}}{\text{Number of cells in mitosis}} = \frac{\text{(Ts) Duration of S phase}}{\text{(Tm) Duration of mitosis}}$$

Thus, the disproportionate increase in mitotic figures compared to labelled cells in the clinical lesion of psoriasis would seem to imply a relative alteration in timing in the cell cycle. One of the possibilities would be if the S phase of the cell cycle was considerably shortened in the active psoriatic lesion, compared to the uninvolved skin. Weinstein & Frost (1968) did find that the S phase in psoriasis was less than in normal skin, but they also reported a shortening of the time taken for mitosis. Another explanation for our findings could be that the duration of mitosis is longer in active psoriasis; in fact Fisher & Wells (1968) did report a lengthening of mitosis from 1½ h in normal epidermis to 4 h in psoriatic epidermis. This result is exactly the opposite to that reported by Weinstein & Frost (1968). At present it is not possible to answer the question of how the various times of the cell cycle are or are not altered in psoriasis compared to uninvolved skin, as there is no knowledge of the cell cycle in uninvolved psoriatic skin.

The increased number of cells labelled with ^{3}H-thymidine in involved psoriatic epidermis was found to be approximately 5 times that in uninvolved psoriatic epidermis and over 10 times that in normal skin. These differences are greater than those reported by Hell & Hodgson (1966) studying similar tissues *in vitro*. However, the number of labelled cells in uninvolved psoriatic epidermis is approximately 2½ times that in normal epidermis, a difference which is similar to that reported by Hell & Hodgson (1966) studying the tissues *in vitro*. Weinstein & Frost (1968), however, did not find any difference between the number of labelled cells in uninvolved psoriatic epidermis and in normal epidermis, using *in vivo* ^{3}H thymidine. If our *in vivo* results are correct and there is an increase in numbers of labelled cells, this is a significant result which must be explained. In addition, it should be remembered that we also found a proportional increase of labelled cells to mitotic figures, compared to normal skin. The first possibility is that the S phase is particularly long in uninvolved psoriatic epidermis. There have already been reports of an S phase of 30 h in rat ear epidermis (Sherman, Quastler & Wimber, 1961), and it could well be that there is a considerable alteration of the length of the S phase. Again, the relative increase of labelled cells could represent polyploidy, which is known to occur in certain malignant cells and even in some normal tissues (Thompson, 1960). Finally, this increase of labelled cells could represent uptake of thymidine by cells not developing polyploidy, but simply synthesizing what has been termed metabolic DNA (Pelc, 1963). This concept, postulated by Pelc, was that cells could synthesize DNA and then break it down again so that the DNA content of the cell remained the same. The answer to what happens in uninvolved psoriatic epidermis can only be ascertained by estimating the components of the cell cycle in this tissue and by estimating the DNA content of the nucleus. Whatever the answer, it does appear that the cell cycle in uninvolved psoriatic epidermis is different to that in normal skin.

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Enzymes of buccal epithelium in psoriasis

HANS HAMMAR

Department of Dermatology, Karolinska Sjukhuset, Stockholm, and Department of Histology,
University of Uppsala, Uppsala, Sweden

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SUMMARY

The normal buccal mucosa was analysed by application of Lowry's microtechniques in thirteen psoriatic patients with guttate skin lesions and in fifteen healthy controls, for the following enzymes: glucose 6-phosphate, glycerol 3-phosphate and glyceraldehyde phosphate dehydrogenases, pyruvate kinase (PK) and NAD and NADP dependent isocitrate dehydrogenases. The material was obtained from superficial and adjacent basal parts of the epithelial rete ridges. Enzymatic activities were higher in basal than in superficial epithelium except for PK in controls. The PK activity was lower in the psoriatic patients than in the controls when the superficial epithelium was compared. No differences were found between the controls and the psoriatic patients with regard to activities of the other five enzymes measured.

In a survey of the literature, Journé (1965) found eighteen cases with psoriatic lesions of the oral mucosa. Kaidbey & Kurban (1971) observed increased labelling of buccal epithelium with ^{3}H-thymidine in a group of psoriatic patients. The question arose as to whether the mucosal alterations found in the psoriatic patients were an indication that psoriasis is a more generalized disease involving epithelia other than the epidermis. There have also been discussions recently concerning psoriatic enteropathy (Shuster & Marks, 1970; Preger *et al.*, 1970; Marks & Shuster, 1971; Summerly & Giles, 1971).

A preliminary survey was therefore undertaken in which patients with guttate psoriasis were selected for assays of some glycolytic and citric acid cycle enzymes in buccal epithelium of normal appearance. The results of the screening are presented in this report.

MATERIAL AND METHODS

Punch biopsies were taken from thirteen patients with psoriasis, without anaesthesia, from macroscopically healthy buccal mucosa at the level of the second premolar tooth. These patients were 16–48 years old (median age 28 years) and had had the disease for 0·5–41 years (median 9 years). The control material consisted of fifteen healthy subjects, of whom three had recently been treated for localized dermatitis, six for leg ulcer, three for urticaria and one for asthma. They gave a negative history of psoriasis, of lichen planus and of recurrent or chronic diseases of the oral mucosa. The controls were 20–78 years old (median age 39 years).

TABLE 1. Assay conditions

Enzyme	Buffer	Substrate	Additives	Volume (μl)	Tissue weight (μg)	Time of incubation (min)	Final volume (ml)	Product measured
1. G6PDH	0·1 M 2-amino-2-methyl-1,3-propandiol§ pH 9·3	4·6 mM glucose 6-phosphate†	0·3 mM NADP 0·5 mM EDTA 1·1 mM $MgCl_2$ 0·05% BPA	1·99	0·20–0·40	15	0·14	NADPH
2. G3PDH	0·1 M triethanolamine† pH 7·6	1·8 mM dihydroacetone phosphate†	0·07 mM NADH 0·05%BPA	1·99	0·20–0·45	60	0·14	NAD
3. GAPDH	25 mM tris (hydroxymethyl) aminomethane pH 8·6	0·3 mM glyceraldehyde phosphate	4 mM disodium arsenate 0·25 mM NAD 1·5 mM EDTA 5 mM mercaptoethanol 0·02%BPA	1·99	0·10–0·25	45	0·15	NADH
4. PK	0·1 M triethanolamine† pH 7·6	1·7 mM phosphoenolpyruvate†	2·3 mM NADH 2·9 mM ADP 19 mM nicotinamide† 84 mM KCl 3·6 mM $MgSO_4$ 3·1 U/ml LDH 0·04% BPA	3·95	0·15–0·30	45	0·14	NAD
5a. ICDH(NAD)	0·1 M tris (hydroxymethyl) aminomethane§ pH 8·0	9·6 mM DL-isocitrate§	0·8 mM NAD 8 mM ADP 8 mM $MgCl_2$ 0·02% BPA	4·49	0·07–0·15	30	—	NADH to be assayed (p. 5b)

TABLE 1. *cont.*

5b. Bioluminescent system	70 mM phosphate Na : K eq. 4 : 1 pH 6·8	NADH 10 μl aliquot added	0·26 mM FMN 8·4 mM ethanethiol§ 2·8 μl myristaldehyde‡ (saturated in methanol) 40 μg A.F. luciferase	56	—	—	0·14	NADH induced flash
6. ICDH(NADP)	0·1 M tris(hydroxymethyl) aminomethane pH 8·0	50 mM DL-isocitrate	0·5 mM NADP 10 mM $MgCl_2$ 0·02% BPA	9·6	0·15–0·40	120	0·14	NADPH

ADP = Adenosine 5′-diphosphate†
A.F. luciferase = Extract of *Achromobacter fischerii*§
BPA = Bovine plasma albumin*
EDTA = Ethylenediaminetetraacetic acid§

FMN = Flavin mononucleotide†
GAPDH = Glyceraldehyde phosphate dehydrogenase, EC 1.2.1.12
G3PDH = Glycerol 3-phosphate dehydrogenase, EC 1.1.1.8
G6PDH = Glucose 6-phosphate dehydrogenase, EC 1.1.1.49

ICDH(NAD) = Isocitrate dehydrogenase, NAD-dependent, EC 1.1.1.44
ICDH(NADP) = Isocitrate dehydrogenase, NADP-dependent, EC 1.1.1.42
LDH = Lactate dehydrogenase†, EC 1.1.1.27
NAD,NADH = Oxidized, reduced nicotinamide adenine dinucleotide†
NADP,NADPH = NAD-, NADH-phosphate‡
PK = Pyruvate kinase, EC 2.7.1.40

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* Armour Pharmaceutical Co., Eastbourne, England: † Boehringer Mannheim GmbH, Germany: ‡ Fluka AG, Buchs, Switzerland: § Sigma Chemical Co., St. Louis, U.S.A.

Preparation of the biopsy material

The biopsies were immediately frozen in isopentane at −86°C, and kept at this temperature in a Dewar vessel until sectioned. The sectioning was performed within 2 days and the further treatment of the material, such as dissection and weighing, was done according to the methods of Lowry (1953) and Lowry, Roberts & Chang (1956) as described earlier (Hammar, Thyresson & Brolin, 1968; Hammar, 1970). The dissected specimens contained the superficial part of the spinous layer and basal part of the rete ridges. The most superficial 1–3 rows of cells were discarded in order to eliminate a possibly contaminated surface and a possibly parakeratinized surface layer. Central parts of the rete ridges were used and the material amounted to 1/3 to 1/5 of the epithelial thickness. Care was taken to use superficial and adjacent basal parts of the same rete ridges.

Enzyme Assays

The assays were carried out according to the principles of Lowry *et al.* (1956) and Lowry, Roberts & Kapphahn (1957), with measurement of the reaction product in a Farrand ratio fluorometer after treatment with strong alkali, and dilution. ICDH(NAD) was assayed by means of a bioluminescent system utilizing a luciferase extract from *Achromobacter fischerii* as described by Brolin *et al.* (1971). The details are presented in Table 1. For each enzyme, time curves and plots of substrate consumption against tissue weights showed linearity for intervals which included the final settings given in Table 1. Optimal pH was obtained for ICDH(NAD) after measurements of the activity in the pH interval 6–10, and for ICDH(NADP) in the pH range 7–9. Both enzymes showed a broad pH curve with an optimum around pH 8·0. The enzymes G6PDH, G3PDH, GAPDH and PK were analysed according to Lowry *et al.* (1961), Brolin & Berne (1967), Velick & Furfine (1963) and Brolin, Berne & Linde (1967), respectively. Appropriate standards and blanks were carried through the entire procedure. Activities were calculated from means of quadruplicates and expressed as moles of substrate converted per kg dry weight per hour of incubation at 38°C. Statistical significance of differences were obtained by '*t*' tests and correlation analyses according to standard procedures (Snedecor & Cochran, 1967). The experimental error was measured on quadruplicates and expressed as a coefficient of variation. It was 19% for G6PDH and PK, 17% for G3PDH, 23% for GAPDH, 20% for ICDH(NADP) and 30% for ICDH(NAD).

RESULTS

The results of the enzyme assays are summarized in Table 2. All of the six enzymes measured showed

TABLE 2. Enzymatic activities of clinically normal buccal epithelium in psoriatic patients and healthy controls. The material consisted of the superficial and the adjacent basal parts of the same rete ridges. Means and their standard error are expressed as moles of substrate converted per hour and kg dry weight at 38°C

	Control			Psoriasis		
	No.	Superficial	Basal	No.	Superficial	Basal
G6PDH	10	3·00 ±0·17	3·58 ±0·26	8	2·78 ±0·36	3·73 ±0·37
G3PDH	10	0·076±0·010	0·168±0·024	9	0·092±0·012	0·173±0·024
GAPDH	13	0·75 ±0·07	1·09 ±0·09	11	0·72 ±0·08	1·09 ±0·12
PK	11	2·52 ±0·16	2·96 ±0·27	10	1·95 ±0·14	2·62 ±0·22
ICDH(NAD)	10	0·083±0·014	0·160±0·026	10	0·090±0·013	0·143±0·027
ICDH(NADP)	9	1·24 ±0·10	1·90 ±0·11	9	1·21 ±0·14	1·98 ±0·18

higher activities in basal than in superficial parts of the buccal epithelium, as judged by '*t*' tests, except for PK in controls ($P < 0{\cdot}05$–$0{\cdot}005$). On comparison of controls and psoriatic patients, no differences were found in the activities of G6PDH, G3PDH, GAPDH and the ICDHs. PK, however, showed lower activity in the superficial epithelium of the psoriatic buccal mucosa than in that of the controls ($P < 0{\cdot}01$).

DISCUSSION

Enzymatic assays of oral mucosa have been sparse and have been mostly concerned with leukoplakias (for references see Alvares *et al.*, 1972). In the homogeneous type of leukoplakia studied by Alvares *et al.* (1972) and Hammar & Alvares (1973), increased activities of the enzymes G6PDH, G3PDH and LDH were evident in superficial spinous layers. Malate dehydrogenase and the ICDHs, on the other hand, displayed decreased activities. The activity of G6PDH was also increased in the normal buccal epithelium of the patients with leukoplakia, which indicates that this enzyme might be useful for predicting an alteration of the epithelium. As shown earlier, G6PDH exhibited increased activity in the border region of a guttate skin lesion (Hammar, 1970). However, no change was found in the buccal mucosa in the psoriatic patients (Table 2).

G3PDH is one link in the glycerol-3-phosphate shuttle transferring reducing equivalents into mitochondria (Lehninger, 1970; van Dam & Meyer, 1971). In a previous study the activity of this enzyme was found to be decreased in the psoriatic skin lesion (Hammar *et al.*, 1968). On the other hand, the mitochondrial enzyme ICDH(NAD) was increased in activity 2·3–2·8 times (Hammar, 1973), and the oxygen consumption was increased 2·9 times (Hammar & Hellerström, 1970), when the assays were made on epidermal material from guttate psoriatic lesions. These changes were not found in the non-involved skin of the psoriatic patients, and in the buccal mucosa there was no sign of such a pattern (Table 2).

The activity of PK was decreased in the psoriatic patients. In the material from leukoplakic lesions, on the other hand, the enzymatic activity was not changed as compared with the control mucosa (Hammar & Alvares, 1973). Since this enzyme catalyses a non-equilibrium step in glycolysis (Krebs, 1969), these findings might indicate that the glycolytic capacity of buccal epithelium in psoriatic patients is different from that in healthy subjects. This decrease in PK activity was the only difference found, however, but the observation justifies further studies concerning epithelial metabolism in psoriasis.

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Atopy in lichen simplex (neurodermatitis circumscripta)

GURMOHAN SINGH

Head, Section of Skin and Venereal Disease, Institute of Medical Sciences, Banaras Hindu University, India

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SUMMARY

A statistical study of 102 patients with lichen simplex lesions and a large group of controls shows a significant association between this disease and a personal and family history of atopic disorders.

This communication compares the prevalence of a personal and family history of atopy in patients with lichen simplex with that in a control group.

MATERIAL AND METHOD

Patients were unselected. A detailed inquiry was made to elicit any history of atopy in the patient or his relatives.

A clinical diagnosis of lichen simplex was made when the patient had lesions on the nape of the neck, ulnar border of the forearm, pretibial area and other sites which are easily accessible to rubbing and scratching. In the present study group the following sites were involved (Table 1).

TABLE 1.

Site involved	No. of patients	Percentage
Neck only	49	48·1
Ulnar border of one forearm	15	14·7
Ulnar border of both forearms	11	10·8
Pretibial skin	8	7·8
Pretibial skin, forearm and neck	6	5·9
Neck and forearm	3	2·9
Neck and leg	3	2·9
Neck and arm	2	2·0
Arm and leg	5	4·9
Total	102	100·0

A random sample of 1000 control subjects from the Skin and Venereal Disease Outpatients Department and the General Medical wards of the hospitals, who had attended for any disease other than psychogenic or allergic, were similarly interviewed (Singh, 1972).

RESULTS

Table 2 shows the incidence of personal allergic manifestations in patients and controls.

TABLE 2. Showing incidence of different allergic manifestations in cases of lichen simplex and controls.

Allergic manifestations	Lichen simplex (102 patients)		Controls (1000 patients)		Z	P
	No.	%	No.	%		
Asthma	16	15·7	64	6·4	2·5206	<0·02
Allergic rhinitis	27	26·5	154	15·4	2·4523	<0·02
Urticaria, recurrent	7	6·9	45	4·5	0·9126	>0·36
Urticaria, evanescent	13	12·7	66	6·6	1·8108	>0·01
Non-allergic	52	50 9	738	73·8	4·58	<0·01
Concomitant (two or more) allergic manifestations	13	12·7	67	6·7		

Statistical analysis

(1) The incidence of asthma and allergic rhinitis in patients with lichen simplex is significantly higher than in controls.

(2) There is no significant difference in the incidence of a personal history of urticaria (either recurrent or evanescent) in the two groups.

(3) The percentage of subjects lacking a personal history of allergy is higher in the controls as compared to patients with lichen simplex. The difference is highly significant.

A family history of atopy was present in forty-five subjects (44·1%). Table 3 shows the family history of atopy in patients with lichen simplex and in controls.

TABLE 3.

Group	No. of subjects studied	No. with positive family history	Percentage
(a) Lichen simplex	102	45	44·1
(b) Control group as a whole	1000	170	17·0
(c) Controls with history of one or more allergic manifestations	313	109	34·8

Statistical analysis

(1) When (a) and (b) are compared by applying the Chi square test, the value of χ^2 is 41·63. Table values of P for one degree of freedom are $<0{\cdot}001$; The difference is therefore highly significant.

(2) On comparing (a) and (c), the Chi square is 2·4626 and the table values of P for one degree of freedom are $<0{\cdot}10$. The difference is not significant.

DISCUSSION

The lesions seen in lichen simplex are similar to those seen in atopic dermatitis (also called Besnier's prurigo and neurodermatitis disseminata). There is, however, a difference in severity and sites of involvement. In atopic dermatitis the lesions are usually bilateral and involve the ante-cubital and popliteal fossae, the neck and other flexural areas. Patients with atopic dermatitis have a high incidence of other atopic manifestations, such as asthma and allergic rhinitis, and there is frequently a very strong family history of atopy.

Morphological similarities between the individual lesions of atopic dermatitis and of lichen simplex suggest that these two entities may in some way be related to each other.

The data analysed indicate that a larger percentage of subjects with lichen simplex had atopic manifestations as compared to controls who were not suffering from atopic disorders. The differences were statistically significant.

The history of atopy in members of the family, which included parents, siblings and children, was positive in 44·1% of patients as compared to 17·0% in controls. These differences were statistically highly significant. The association with atopy is further supported by comparing the incidence of atopy in families of patients with lichen simplex with that of the group of control subjects who had given a positive personal history of one or more atopic manifestations; they were not significantly different.

These results differ from those of Carr, Berke & Becker (1964), who made the only other investigation on this subject of which we are aware. They studied fifty-three subjects with lichen simplex and found no significant increase in the atopic background as compared to a control population. Unfortunately, they gave no criteria for the diagnosis of lichen simplex. A diagnosis of lichen simplex is made by some dermatologists only when the patient gives no history of atopy. If a clear history of atopy (personal or familial) is elicited, the diagnosis is recorded as atopic dermatitis. Sulzberger *et al.* (1961) also have stated that the absence of other findings of atopy helps to distinguish cases of lichen simplex from atopic dermatitis. If the lack of history of atopy is taken as a criterion for the diagnosis of lichen simplex, it is evident that the cases to which he applies this diagnosis are those without an atopic background. This deliberate though unconscious selection of patients may explain the findings of Carr *et al.* (1964).

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On the persistence of positive patch test reactions to balsam of Peru, turpentine and nickel

J.C.A.TELINTUM AND J.P.NATER

Department of Dermatology, State University, Groningen, The Netherlands

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SUMMARY

A group of 105 patients with positive patch test reactions to turpentine, balsam of Peru or nickel were re-examined after periods of 2–15 years.

Of twenty-eight patients who had shown positive patch test reactions to turpentine eighteen gave negative reactions; of twenty persons formerly positive to balsam of Peru ten had become negative. Of fifty-seven patients who had formerly been positive to nickel eighteen gave negative reactions. The implications of these findings in relation to the time factor and the possibility of avoidance of the allergen are discussed.

The course of acquired epidermal sensitivity is controversial. Formerly, several authors thought that a sensitivity once acquired was unlikely to be lost. However, it was thought possible that a sensitivity might disappear, especially when contact with the sensitizer could be avoided (Bonnevie, 1939).

It is not unlikely that data concerning the duration of contact sensitivity will gain increased significance, now that the capacity of the organism to develop a contact sensitivity has become an important subject of immunological research. The questions arise, whether a patient who appears to have lost a formerly acquired contact sensitivity (e.g. to DNCB) has lost his positive patch test reaction as a consequence of a change in his cellular immune system or whether the loss of this reaction need imply no impairment of his cellular immune response.

The existing literature on the persistence of positive patch test reactions is summarized in Table 1. Several authors (e.g. Nielsen & Bang, 1954) included not only sensitizing substances but also irritants, such as soap, in their investigations. We have restricted our survey to the data they provided on some unquestionable allergens.

There may be a difference between the persistence of a spontaneous and of an experimental sensitivity (Kanof & Rostenberg, 1941). However, data on the persistence of experimental sensitivities in human subjects are scarce. Wedroff & Dolgroff (1935) sensitized normal subjects with DNCB. They found that more than half of their cases had lost their positive reaction spontaneously within a year. This loss or reduction of sensitivity to DNCB was confirmed by White & Baer (1950). From their study it appeared that in twenty of 124 subjects hypersensitivity to DNCB was no longer demonstrable 'several weeks' after sensitization. Meneghini *et al.* (1967) made the same observation on the persistence

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Requests for reprints: J.P. Nater, Department of Dermatology, University Hospital, Oostersingel 59, Groningen, The Netherlands.

TABLE I.

Author	Allergen	Interval in patch testing (years)	Total no. of patients	Diminished on re-patch testing	Negative on re-patch testing	Diminished or negative *without* avoidance of allergen	Diminished or negative *with* avoidance of allergen
Gomez-Orbaneja 1938	Turpentine	1–3·5	?			Diminished 15% Negative 3%	Diminished 13% Negative 59%
Morgan 1953	Nickel	1·5–11	54		43%	80%	20%
Bülow 1954	Balsam of Peru	Average 4·2	18		16%		
	Turpentine		12		66%		
	Nickel		9		22%		
Nielsen & Bang 1954	Balsam of Peru	Average 13·5	9	33%	44%		
	Turpentine		29	6%	62%		
	Nickel		11	36%	18%		
Fisher & Shapiro 1956	Nickel	2–5	40		10%		
Marcussen 1959	Nickel	5·5–7·6	27	True pos. 8%	True pos. 4%		
			11	False pos. 0%	False pos. 80%		
Lakaye & Lapière 1961	Turpentine	1–6	?		6%		
	Chromate				25%		
	Para				74%		
Rhodes & Warner 1966	Nickel	2	21		38%		

of DNCB sensitization, but stated that sensitivity to NDMA (N : N-dimethyl-*p*-nitrosoaniline) rarely declined. They supposed that subjects sensitized to NDMA are more liable to come into exogenous contact (dyes, drugs, anaesthetics, cosmetics) or endogenous (dyes for foods, drugs) with similar chemical substances of the paragroup. Substances related to DNCB are much less often encountered in daily life.

The relative scarcity of information on the persistence of a positive patch test prompted the study which is reported in the present communication.

The object of the investigation was:

(1) To ascertain whether patients were sufficiently informed about the causes of their allergic contact dermatitis.

(2) To attempt to follow the course of formerly established allergy, by re-patch testing after an interval of several years.

(3) To try to ascertain whether attempts by patients to avoid contact with an allergen had resulted in a significant decrease or disappearance of a positive patch test.

MATERIALS AND METHODS

A questionnaire was sent to a group of patients who had been patch tested in our clinic over a period of at least 2 up to 15 years ago.

According to the files, these persons had formerly been diagnosed as suffering from a contact dermatitis caused by turpentine, balsam of Peru or nickel. Only patients who had scored at least two-plus reactions during the first patch test series were included in our investigation. In the questionnaire patients were asked if they could remember to which substances they had been found to be allergic and if they had attempted to avoid the allergen. They were asked to visit the clinic to be patch tested again. Though some of these persons had formerly been found to react also to other materials only the course of the patch test reaction to the substance (either turpentine, balsam of Peru or nickel) which had been considered to be the cause of the contact dermatitis was studied.

The following groups of patients were patch tested again.

(A) Twenty-eight persons (seventeen men and eleven women) formerly diagnosed as contact dermatitis caused by turpentine. The average interval before repeat patch testing was 7 years.

(B) Twenty persons (eight men and twelve women) formerly diagnosed as contact dermatitis caused by balsam of Peru. The average interval before repeat patch testing was 5 years.

(C) Fifty-seven persons (ten men and forty-seven women) formerly diagnosed as a contact dermatitis caused by nickel. The average interval before repeat patch testing was 7 years.

The average age of all the patients was 48 years.

Patch tests were performed with Scandinavian Silverpatches® on the upper arm, and were read according to the methods recommended by the International Contact Dermatitis Research Group and evaluated after 48 and 72 h. Patients were asked to telephone us should patch tests react after a week or more.

The following materials were used:

(1) Turpentine peroxide 0·3% in olive oil as advised by the International Contact Dermatitis Research Group and obtained from Trolle Lassen, Denmark.

(2) Oil of turpentine 25% in oil of sesame. This was the original material used in former years for patch testing. The same batch of oil of turpentine, which had been stored in a cool and dark place, was still available.

(3) Balsam of Peru in a concentration of 25% in soft yellow paraffin, the same as had been used formerly.

(4) Nickel sulphate 5% in soft yellow paraffin, as in the earlier patch tests.

RESULTS

The question as to whether these 105 patients remembered which materials had to be avoided was answered as follows:

Twelve of twenty-eight patients (42%) with positive patch test reactions to turpentine defined their allergen incorrectly; eleven of twenty persons (55%) allergic to balsam of Peru were ignorant of this fact. As far as nickel sulphate was concerned, six of a total of fifty-seven patients (12%) could not remember the offending agent. Re-patch testing showed that ten of twenty-eight patients in the turpentine group still reacted positively (36%), whereas ten of twenty patients in the balsam of Peru group were still positive (50%) and thirty-nine of fifty-seven patients (68%) remained positive to nickel. The combination of these patch test results with the results of the questionnaire made it clear that twenty-one of thirty-five patients who tried to avoid the allergen (turpentine, balsam of Peru or nickel sulphate) still reacted positively; fifteen of thirty patients who defined the allergen correctly but who were less conscientious in avoiding it still reacted positively, whereas twenty-three of forty persons who could not avoid the allergen showed positive patch test reactions.

Separate data on turpentine, balsam of Peru and nickel are presented in Tables 2, 3 and 4.

TABLE 2. Patch test results with turpentine

	No. of patients	
	Positive	Negative
Allergen correctly defined and avoided	2	6
Allergen defined correctly; avoidance doubtful	2	5
Allergen incorrectly defined or not avoided	6	7
Total	10	18

TABLE 3. Patch test results with balsam of Peru

	No. of patients	
	Positive	Negative
Allergen correctly defined and avoided	3	1
Allergen defined correctly; avoidance doubtful	1	2
Allergen incorrectly defined or not avoided	6	7
Total	10	10

TABLE 4. Patch test results with nickel sulphate

	No. of patients	
	Positive	Negative
Allergen correctly defined and avoided	16	7
Allergen defined correctly; avoidance doubtful	12	8
Allergen defined incorrectly or not avoided	11	3
Total	39	18

Another point of interest was to assess the possible influence of the time interval between the first and second patch testing on the persistence of the positive tests. These data are given in Table 5. There appears to be no clear correlation between this time interval and the persistence of positive reactions.

In one case a difference was noted in the patch test reactions to oil of turpentine and to turpentine peroxide. One patient reacted + + only to turpentine peroxide and not at all to oil of turpentine.

TABLE 5. Influence of time on the persistence of positive patch tests

	Interval between patch tests (years)				
	2–4	5–8	9–12	13–15	Total
Patch tests positive	7	39	6	7	59
Patch tests negative	16	17	8	5	46
Total	23	56	14	12	115

Terpstra's statistical trend test: $T = -1{\cdot}1278; P = 13\%$.

This patient was scored as positive. One patient tested with nickel sulphate showed no reaction after 48 and 72 hr, but 1 week later the reaction became positive. This patient was scored in our data as negative.

DISCUSSION

A first conclusion from our observations is that 64% of the persons formerly allergic to turpentine, 50% of those allergic to balsam of Peru and 32% of those allergic to nickel had lost their positive patch test reactions, when they were re-tested after an interval of 2–15 years. These results differ from these recorded in the literature (Table 1). It must be mentioned, however, that several studies comprised only small groups of patients, a factor which may influence the significance of the results obtained.

In one of our patients, as the positive patch test reaction to nickel sulphate developed after 1 week, it is possible that active sensitization by the patch test had occurred.

A second conclusion from our observations is that a sizeable proportion of the patients with positive patch test reactions to turpentine or balsam of Peru did not know which substance they had to avoid. Most persons allergic to nickel knew that they had to avoid this material, or, more generally, 'metal'. These figures emphasize the importance of taking sufficient care to inform each patient at the end of an allergic investigation. The presentation of an allergy passport with sufficient information about the proven allergy is apparently necessary, especially in the case of allergens rather difficult for laymen to remember. This is so, despite our third conclusion that our results do not establish with any certainty that avoidance of the allergen has a decisive influence on the persistence of a sensitivity once acquired. A relatively large number of patients, seventeen of forty (42%), who did not attempt to avoid contact with the specific allergen, subsequently lost their formerly positive patch test reactions. The existing literature on the influence of the avoidance of contact with an allergen on the persistence of a hypersensitivity is scanty and contradictory.

Gomez-Orbaneja & Barrientos (1938) mentioned a decrease in positive patch test reactions when the allergen is avoided. The observations of Morgan (1953) lead to an opposite conclusion (Table 1). In our opinion, in spite of good intentions on the part of the patient, it is hardly possible nowadays to avoid contact with nickel or nickel-containing alloys. In the cases of turpentine and balsam of Peru, related compounds may be encountered in unexpected ways in daily life. It is known that numerous compounds and natural products possess immunochemical relationships with the individual constituents of balsam of Peru (Hjorth, 1961). These are facts of which most patients are ignorant. Findings based on the alleged successful avoidance of contact may therefore not be entirely reliable. This is possibly the explanation of the conflicting results reported by Gomez-Orbaneja and Morgan.

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Collagen degradation in cultured keloid and hypertrophic scar tissue

J.P.MILSOM AND R.D.P.CRAIG

Departments of Medicine and Plastic Surgery, University Hospital of South Manchester,
Nell Lane, Manchester M20

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SUMMARY

In order to study collagen catabolism of normal human skin, keloid and hypertrophic scars, explants of these tissues have been cultured for periods of up to 10 days. All three tissues released a similar amount of neutral collagenase activity into the culture medium with maximal yield between days 3 and 7, and the collagenase from scar tissues was found to be identical to the normal skin enzyme with regard to its inhibition by EDTA, cysteine and human serum. The major site of collagenase production in keloid specimens appeared to be, as in normal skin, the upper dermal or epidermal layer, with minimal production occurring in the lower fibrous or nodular areas.

Prior to culture the collagen content of each tissue was found to be similar, with approximately 1% of the total being acid-soluble. During the culture period considerable amounts of insoluble tissue collagen were degraded in all three tissues, as judged by the release of hydroxyproline into the culture medium.

These results suggest that the persistence of keloids and hypertrophic scars is attributable neither to an inability of the tissues to produce an active collagenase molecule nor to any resistance of the tissue collagen to degradation.

It is well-known that certain wounds tend to produce raised, unsightly scars which persist long after the normal remodelling processes might have been expected to remove them. Most biochemical studies undertaken to determine the cause of these keloids and hypertrophic scars have produced inconclusive results (e.g. Hoopes, Chi-Tsung & Im, 1971; Bazin *et al.*, 1970; Psillakis *et al.*, 1971). However, histological differences in the appearance of their collagen fibres as compared with those of normal skin (Blackburn & Cosman, 1966; Holmstrand, Longacre & De Stefano, 1961) suggest that an error may be present either in the synthetic or in the degradative phase of collagen metabolism. It is with the latter that this study is concerned.

If the excess collagen of hypertrophic scars and keloids is the result of the failure of collagen to be degraded, two explanations seem possible. First, abnormal collagen which is resistant to the normal breakdown mechanisms, may be present within the scars or, secondly the activity of one of the major enzymes associated with collagen degradation may be absent from the tissue, whether from deficient synthesis, lack of activation of a proenzyme or absence of a necessary cofactor.

The existence of a mammalian enzyme able to degrade collagen at a physiological pH and temperature is now well established. Although it is not directly extractable from tissues, culture techniques have shown that this neutral collagenase is produced by skin (Eisen, Jeffrey & Gross, 1968), healing wounds (Grillo & Gross, 1967) and a variety of other tissues. Recent work (Woolley *et al.*, 1973), showing the ability of human collagenase to degrade insoluble polymeric collagen, further emphasizes its probable importance in *in vivo* collagen catabolism. The present work was undertaken, first, to investigate the neutral collagenase activity of hypertrophic scars and keloids under culture conditions established for normal skin and, secondly, to determine the susceptibility to degradation of the collagen molecules within these tissues.

Although collagenolytic activity in keloid explants has been demonstrated by lysis of reconstituted gels (Ryan & Peacock, 1967), no quantitative comparisons of activity with that of normal skin have yet been attempted. One of the problems encountered is that of a suitable parameter upon which to base such comparisons. In this work, we have chosen to use glucose uptake by the tissue in culture as an index of the number of viable cells cultured, since cell viability has been shown to be prerequisite for the production of collagenase (Eisen, 1969). As the ratio of extracellular to intracellular material is far higher in hypertrophic scars and keloids than in normal skin, this method might be expected to provide a more accurate basis for quantitative comparisons than those based on wet and dry weights of tissue cultured.

MATERIALS AND METHODS

Tissue culture

Specimens of normal skin, hypertrophic scars and keloids were obtained from a variety of body locations during general surgical and plastic surgical operations. The donors were of either sex and varied in age from 3 to 77 years. Hypertrophic scar and keloid specimens were generally the results of burns or other lesions, and ranged in duration from 5 months to 6 years. After removal of subcutaneous fat, the tissue was cut into pieces approximately 4–6 mm in greatest dimension, and washed five times

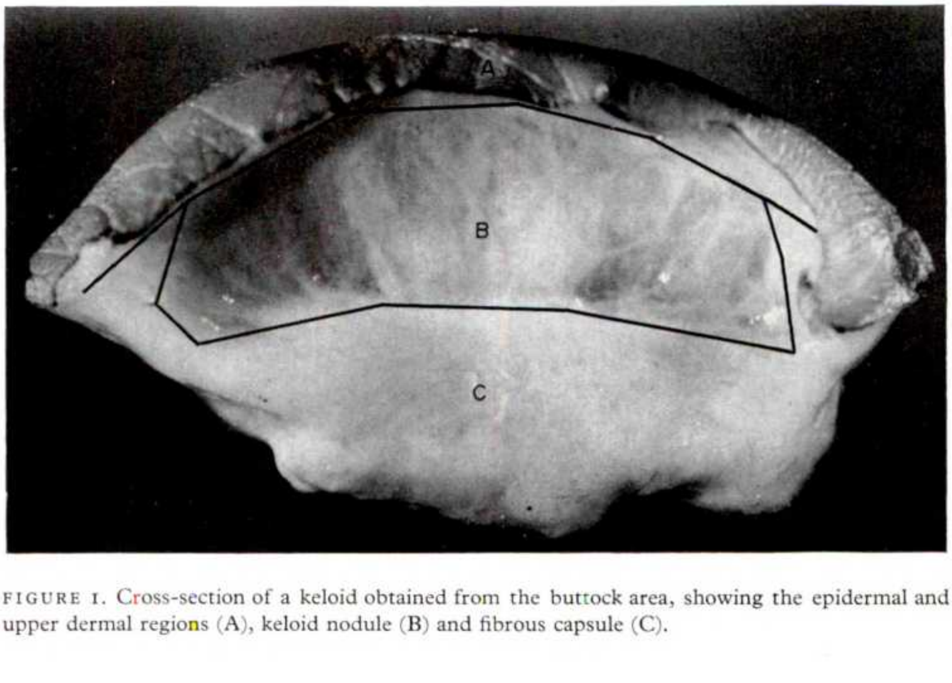

FIGURE 1. Cross-section of a keloid obtained from the buttock area, showing the epidermal and upper dermal regions (A), keloid nodule (B) and fibrous capsule (C).

with Dulbecco's modified Eagle's medium (Wellcome Reagents Ltd) containing 50 units/ml of benzylpenicillin BP (Glaxo Laboratories, Greenford) and 50 μg/ml each of streptomycin sulphate BP (Dista Products, Liverpool) and Nystatin (E.R.Squibb, Liverpool). The tissue was then transferred to disposable plastic flasks (Falcon Plastics) containing 4·5 ml Dulbecco's modified Eagle's medium, and cultured at 37° C in an atmosphere of 95% O_2 and 5% CO_2 for 8–10 days. Culture medium was routinely changed at 24 h intervals and stored at −20° C after the addition of 20 mM Tris and 2 mM $CaCl_2$, pH 7·6.

In certain large keloids obtained from the buttock region, the keloid tissue was sufficiently demarcated to permit its separation by careful dissection (Fig. 1). Cultures were set up of tissue from specific areas of the keloid in an attempt to localize the region of collagenase production.

Assay of culture medium

(a) *Collagenase.* This was performed by a method (Nagai, Lapière & Gross, 1966) which measured the release of soluble ^{14}C-glycine-containing peptides from native, thermally reconstituted collagen fibrils prepared from guinea-pig skin.

Collagenase activity was expressed as μg collagen released into solution per 1 ml of culture medium per 6 h incubation.

Inhibition of collagenase activity. In order to determine the response of the keloid and hypertrophic scar enzymes to known inhibitors of the normal skin collagenase, assays were performed in the presence of varying concentrations of cysteine, EDTA and normal human serum.

(b) *Glucose.* In order to determine the glucose uptake by the tissue, the concentration remaining in the medium was determined by a method (Hultman, 1959) in which 3 ml *o*-toluidine reagent (Sigma) was mixed with 25 μl of culture medium and incubated in a boiling water bath for 8 min. After cooling, the glucose concentration was determined from the extinction at 620 nm.

The glucose uptake of the tissue in culture was expressed as a percentage of the initial glucose concentration in the Dulbecco's modified Eagle's medium (4.5 mg/ml).

Relationship of glucose uptake to quantity of tissue cultured. Different quantities of each of certain specimens of normal skin, keloid and hypertrophic scars were cultured in separate flasks. On the second day of culture the medium was removed and the tissue washed free of absorbed medium, dried and weighed. The glucose uptake from the medium was then related to the dry weight of tissue in the flask for each quantity of explant cultured.

(c) *Hydroxyproline.* This was determined by a modification of Woessner's method (Woessner, 1961). The possible complication of interfering substances in the culture medium was overcome by the use of an internal hydroxyproline standard in each test sample. Hydroxyproline in the medium was expressed as μg/ml.

Estimation of soluble tissue collagen. In all three types of skin, tissue prior to culture was used for the determination of acid-soluble collagen content. This was performed by shaking the previously washed and minced tissue in 0·1 N acetic acid for 48 h at 4° C. The supernatant was then removed by centrifugation. The residue was then washed, dried in a 37° C oven and weighed. Hydroxyproline estimations were performed on both the supernatant and the residue.

RESULTS

Tissue culture

Fig. 2 shows the appearance of collagenase activity in the medium of a typical culture of normal skin, in which enzyme activity appeared after day 2 and reached a maximum between days 4 and 6. Collagenase activity was detected in the medium of all specimens of normal skin, hypertrophic scar and keloid cultured, the patterns of production being essentially similar.

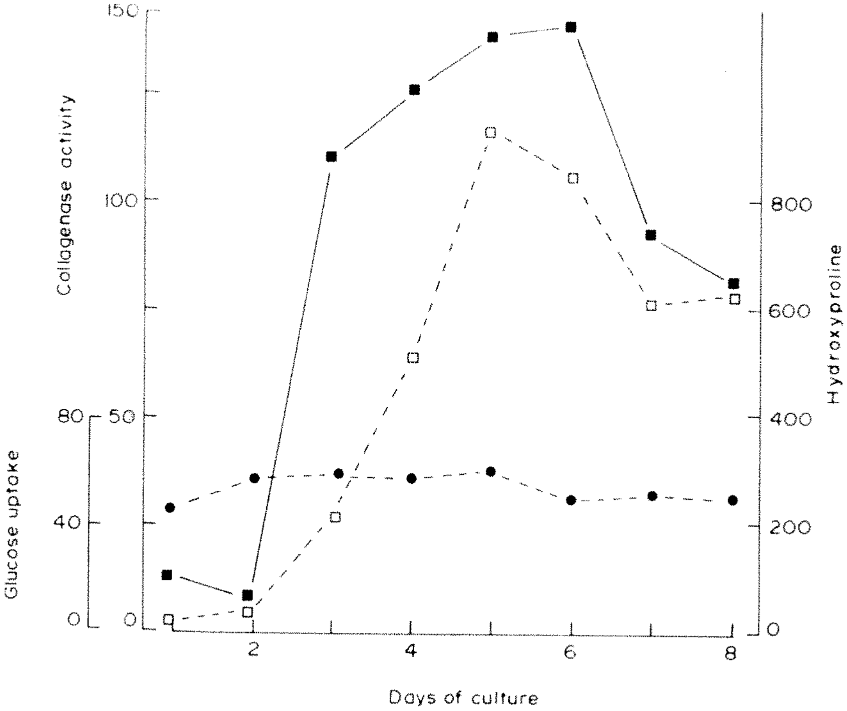

FIGURE 2. Collagenase activity (■——■) and hydroxyproline concentration (□ – – – □) present in the culture medium as a function of the period of culture of a typical specimen of normal skin tissue. Tissue viability is expressed as percentage removal of glucose from the medium (● – – – ●).

It can also be seen from Fig. 2 that degradation of collagen within the explants, as judged by the appearance of hydroxyproline in the culture medium, corresponded closely with the release of collagenase into the medium. Again, this was true for all specimens of normal skin, hypertrophic scar and keloid cultured. At present we are unable to establish any clear quantitative relationship between the concentrations of collagenase activity and of released hydroxyproline in the culture media of these tissues.

All specimens of normal skin, hypertrophic scar and keloid remained viable for at least 10 days, as judged by their glucose uptake, which remained almost constant after day 2 of culture.

Glucose uptake as a function of weight of tissue cultured

Fig. 3 represents data obtained from the culture of varying amounts of a specimen of normal skin, in which the day 2 glucose uptake is proportional to the dry weight of tissue cultured up to a 70–80% uptake, corresponding to a flask loading of 35–40 mg dry weight. This suggests that the glucose uptake is directly dependent upon the number of cells in culture. Although data plotted for other tissue specimens reveal a similar linear relationship, large differences are obtained in the degree of proportionality, which can probably be explained by variation in viability and in the amount of extracellular material present. It would appear from this that glucose uptake provides a more accurate basis than tissue dry weight for the comparison of cellular functions between different samples.

Collagenase production by different types of scar tissue

The total amount of collagenase produced over 8 days of culture was computed for each of sixteen

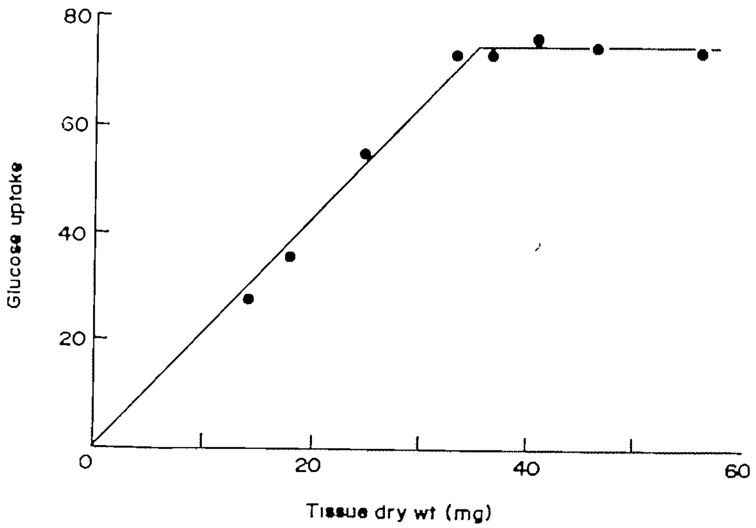

FIGURE 3. Percentage removal of glucose from culture medium as a function of dry weight of tissue cultured. Both parameters were determined on the second day of culture of varying quantities of a specimen of normal skin tissue.

specimens of normal skin, eleven specimens of hypertrophic scar and eight specimens of keloid, and related to the mean daily glucose uptake of the tissue. For each specimen the datum employed was the average obtained from several flasks. Despite the widely differing ages of patients and the different ages and body locations of the specimens, no statistically significant differences were observed in the capacity of the three types of skin to produce collagenase (Student's '*t*' test).

Inhibition studies

The effects of different concentrations of EDTA, cysteine and normal human serum upon the activity of collagenase in the medium of normal skin, hypertrophic scar and keloid are shown in Table 1. It can be seen that inhibition of enzyme activity occurs to the same extent, suggesting identity of the three enzymes.

TABLE 1. Per cent inhibition of collagenases by EDTA, cysteine and serum

	Normal skin	Scar	Keloid
EDTA			
1 mM	87	72	75
5 mM	97	84	94
Cysteine			
1 mM	24	25	26
10 mM	90	84	87
Serum			
1:4000	54	37	42
1:1000	86	85	74
1:200	97	93	89

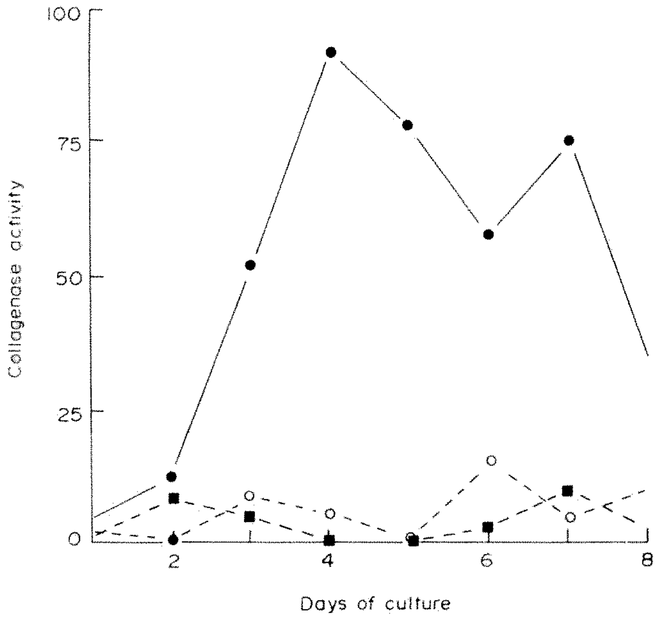

FIGURE 4. Appearance of collagenase activity during culture of separate regions of buttock keloid denoted in Fig. 1 as A (●———●), B (○ - - - ○) and C (■ - - - ■).

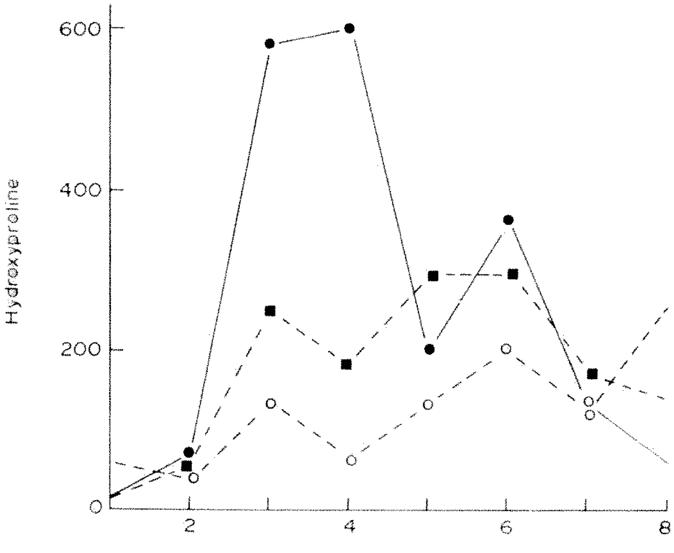

FIGURE 5. Appearance of hydroxyproline during culture of separate regions of buttock keloid denoted in Fig. 1 as A (●———●), B (○ - - - ○) and C (■ - - - ■).

Source of collagenase in keloids

In Fig. 4 is shown the profile of collagenase activity in medium obtained from cultures of the three separated regions of keloid tissue. It is apparent that the major site of collagenase production is that region comprising the epidermis and the top layer of the dermis, and this is reflected in the higher level of hydroxyproline released into the medium by this region (Fig. 5).

Extraction of tissue collagen

The amounts of acid-extractable and insoluble hydroxyproline of normal skin, hypertrophic scar and keloid tissues are presented in Table 2. A considerable variation is observed between individual specimens, as indicated by the high standard deviations. However, it is apparent that hypertrophic scar and keloid tissues resemble normal skin in terms of both their soluble and insoluble hydroxyproline content, the differences between them being statistically insignificant.

TABLE 2. Hydroxyproline content of normal and abnormal skin tissue

	% acid-extractable hydroxyproline		µg insoluble hydroxyproline per mg dry wt of tissue	
Hypertrophic scar	1·99 ± 0·90*	$P>0{\cdot}05$	99·9 ± 29·6	$P>0{\cdot}05$
Normal skin	1·55 ± 1·06		87·7 ± 14·1	
Keloid	1·58 ± 0·82	$P>0{\cdot}05$	106·8 ± 19·0	$P>0{\cdot}05$

* Results are expressed as mean ± standard deviation for a minimum of five experiments.

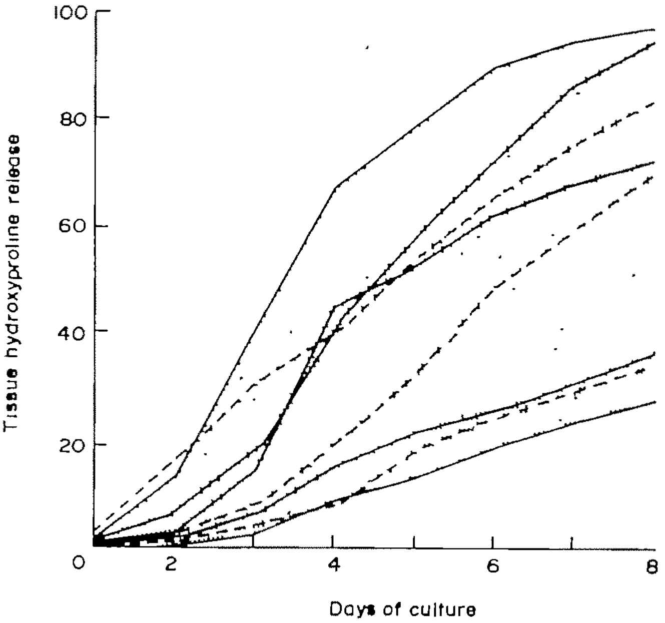

FIGURE 6. Release of tissue hydroxyproline into the medium during culture of normal skin and of scars. The concentration of hydroxyproline accumulated in the medium is expressed as a percentage of the initial tissue hydroxyproline concentration. Results are presented for a number of individual cultures of keloid (– – – – –) and hypertrophic (———) scars, the range of results obtained from sixteen normal skin cultures also being indicated (stippled area).

Breakdown of insoluble tissue collagen during culture

High levels of hydroxyproline have been found in the medium of all types of tissue cultured (e.g. see Fig. 2). This must be derived from the original tissue collagen, as preliminary experiments have shown that no collagen synthesis occurs under the conditions employed in culture. From a knowledge of the amount of hydroxyproline present in the tissue initially, the hydroxyproline released into the medium during culture may be expressed as a percentage of the original total tissue hydroxyproline. The results of this are depicted in Fig. 6 for a number of cultured tissues, including sixteen specimens of normal skin.

It can be seen that, during the culture of normal skin, between 20 and 100% of the initial tissue hydroxyproline may be released into the medium. The culture of keloid and hypertrophic scar also results in tissue hydroxyproline release within the same range. Since it has already been shown that prior to culture soluble collagen comprises only approximately 1% of the total (Table 2), the majority of the hydroxyproline appearing in the medium must be due to the breakdown of originally insoluble collagen.

Susceptibility of residual collagen to collagenase

In order to determine the possible resistance of any residual insoluble collagen to collagenase action, the tissue was retained from two examples each of hypertrophic scar and keloid from which tissue hydroxyproline release during culture was low. To each was added partially purified collagenase (Woolley *et al.*, 1973) obtained from normal skin cultures. After incubation at 37° C for approximately 36 h, greater than 90% of the remnant tissue hydroxyproline was found to have been released into the supernatant in all cases.

DISCUSSION

Using *in vitro* culture conditions previously extensively applied to normal skin, we have shown that viability of explanted scar tissue remains good for up to 10 days, as judged by glucose uptake. Neutral collagenase is released into the culture medium of hypertrophic scars and keloids following a time course similar to that of normal skin and, with respect to its susceptibility to known inhibitors of collagenases, the enzyme appears identical with that produced by normal skin. When compared with normal skin, the capacity of the cultured pathological tissues to produce collagenase appears quantitatively similar. Extraction of hydroxyproline from the scars has shown that the percentage of the acid-soluble component is of the same order as that obtained for normal skin. If it is assumed that scar and normal skin collagens contain the same percentage of hydroxyproline, it would appear that no gross difference exists between the proportion of soluble and insoluble collagen in the two tissues.

During culture of scar tissues, hydroxyproline is found in the medium, as in cultures of normal skin. Quantitatively, the total hydroxyproline recovered from the medium is much greater than that present in the initial acid-extractable portion of the tissue collagen, showing that insoluble polymeric collagen is being degraded. Incubation of tissue remaining after culture with normal skin collagenase results in solubilization of the remaining insoluble collagen.

These results show that, under our experimental conditions, hypertrophic scars and keloids neither lack the ability to synthesize neutral collagenase in amounts similar to normal skin nor contain collagen resistant to the action of this enzyme. Moreover, since the breakdown of endogenous collagen in the explanted scars proceeds at a rate similar to that in normal skin, scar collagen is not unduly resistant to degradation, irrespective of the precise role of neutral collagenase in this process. Thus, it might be concluded that the failure of these scars to resorb under normal remodelling conditions is not due to the presence of an abnormally resistant collagen or to the failure of normal resorptive enzyme systems. Such a conclusion is not entirely justified, however, since removal of the tissue from its *in vivo* environment might remove it from important influences operative in the body. For instance, it is well established that there are natural inhibitors of collagenase in serum, the most important of which is α_2-macroglobulin (Eisen, Block & Sakai, 1970; Abe & Nagai, 1972), and it is probable that within tissues themselves inhibitors of collagenase are also present. Although preliminary observations suggest that serum inhibitory activities of normal subjects and of those with keloids do not differ, the existence of local differences in the amount of tissue inhibitors has yet to be excluded.

Moreover, since recent work (Harper, Block & Gross, 1971; Harper & Gross, 1972) suggests the presence within tissue of a collagenase pro-enzyme which becomes activated under appropriate conditions, it remains possible that *in vivo* this process is suppressed and local enzyme activity within the scars correspondingly diminished.

The experiments with cultures of separated keloid layers indicate that the upper dermis is the major site of collagenase production, as in normal skin (Eisen, 1969). The lower layers of keloid were virtually devoid of enzyme and it is possible, therefore, that some degree of restraint of enzyme movement from the site of synthesis to the site of action may operate *in vivo*.

Further work using immunofluorescence techniques to detect inhibitors and enzymes within the tissue is required to probe further into these possibilities.

ACKNOWLEDGMENTS

The authors wish to thank Professor J.M.Evanson and Dr D.E.Woolley for their advice and criticisms. The work was supported by a grant from the South Manchester Regional Hospital Board.

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British Journal of Dermatology (1973) **89**, 645.

Comment

HIGHLIGHTS OF THE TENTH INTERNATIONAL LEPROSY CONGRESS, BERGEN, NORWAY, 13–18 AUGUST 1973

The most recent break-through in the experimental field has been the discovery in the U.S.A. that the nine-banded armadillo is proving a valuable animal model for the study of *Mycobacterium leprae*. One-third of inoculated animals develop disseminated infection, similar to lepromatous leprosy in man, within 30 months when inoculated intravenously, and within 37 months when the dermal route is used. Among the biological features peculiar to the armadillo, and particularly relevant to leprosy, are the following: (1) low body temperature (32–35° C), (2) long life-span (12–15 years), and (3) regular production of litters of monozygous quadruplets.

So far no inoculated armadillo has developed leprosy of tuberculoid or of borderline type. This is where the mouse provides a useful counterpart for unravelling the immunological complexities of leprosy, as the mouse (if not immunologically suppressed) is capable of producing cell-mediated immunity to *Myco. leprae*, whereas the armadillo is not. Another important aspect of lepromatoid leprosy in the armadillo is that it provides a rich source of supply of leprosy bacilli for research centres all over the world.

As regards cultivation of *Myco. leprae in vitro*, recent evidence was produced of a limited multiplication within macrophages derived from human peripheral blood, but the applicability of this method is restricted by the limited survival of the host cells.

A number of papers dealt with immunological responsiveness to *Myco. leprae* by the methods of lymphocyte transference, macrophage migration inhibition, and delayed skin hypersensitivity, and demonstrated a continuous decrease from strong responses at the tuberculoid end of the leprosy spectrum to virtually negative responses at the lepromatous end. The inference is that clinicopathological manifestations of leprosy reflect the patient's cell-mediated immune responses to *Myco. leprae*. One paper, of particular interest to immunologist and clinician alike, described six fatal cases of strongyloidiasis (a nematode infection of the bowel), in patients suffering from lepromatous leprosy who had received long-term steroid therapy. Presumably, hyperinfection resulted from impaired cell-mediated immunity further intensified by steroids.

There was general agreement that the two types of lepra reaction are immunologically distinct, being an immune complex syndrome in lepromatous leprosy, but due to a rapid shift in cell-mediated immunity in the borderline and tuberculoid types.

In the section on therapy, it was accepted that dapsone has maintained its pre-eminent position, but the question of increasing drug resistance is beginning to pose a serious problem. There was no unanimity on optimum dosage, but there was general agreement on continuing treatment of lepromatous leprosy for life. Several papers reported satisfactory results with a long-acting acetyl derivative of dapsone given by intramuscular injection once every 75 days (DADDS, acedapsone, Hansolar), not only in therapy but also in chemoprophylaxis, in spite of the very small daily dose of dapsone liberated (about 2·5 mg). There were a number of papers on the good antileprosy effect of clofazimine (Lamprene, B663), and on its value in controlling reaction in lepromatous leprosy (ENL), including leprosy neuritis. Its high cost, and the red-brown pigmentary changes it causes in light skins, mitigate against its use in uncomplicated lepromatous leprosy. A new analogue of clofazimine (B1912) has been synthesized; it is cheaper to make, and experiments with mice have shown it to have the same order of activity. Rifampicin received a good deal of attention, and its rapid bactericidal action was confirmed in all reports; the rapid killing of *Myco. leprae* prevents the slow release of intracellular antigens of the bacilli, thus explaining the reduced incidence of ENL reaction. Caution was advised in using this antibiotic in borderline leprosy because of the danger of crippling upgrading reactions. In general, it was felt that its high cost and limited availability would restrict its use to selected cases of lepromatous leprosy, in which it is used for only a limited period in view of experiments which have shown that, after 2 weeks of treatment, bacilli from the skin fail to produce a foot pad infection in mice. A new approach to treating the lepromatous patient is by means of transference of cell-mediated immunity, and various methods were discussed, such as injecting lymph node cells or lymphocytes collected from tuberculoid donors; but this work is only in its infancy and conclusions are impossible at this stage. As regards surgical treatment, there was much discussion on the management of leprous neuritis, and on the consequences of neuritis, such as hand or foot deformity and trophic ulceration. In the section of ophthalmology, the importance of diagnosing and treating silent, painless chronic iridocyclitis was stressed.

In the section of pathology, new light was thrown on the mechanism of nerve damage. In tuberculoid leprosy, the cell-mediated immune response against *Myco. leprae* destroys both bacilli and the Schwann cells that contain them, whereas in the lepromatous type bacilli multiply freely in Schwann cells and in perineural cells; their presence in perineural cells impairs the functional efficiency of these cells, allows leakage of extracellular fluid constituents into the endoneurium, thus causing impairment of nerve function, which is probably reversible. In an attempt to repair the perineurium, Schwann cells take on the form and function of perineural cells and form multiple layers of 'pseudoperineurium', and this slow loss of Schwann cells is the major cause of irreversible nerve damage in lepromatous leprosy.

The frequency of striated muscle infection in leprosy was first noted in mouse foot pad infection, and this has stimulated a number of workers to carry out muscle biopsies on patients; these have shown *Myco. leprae* within and between muscle bundles, but not in large numbers.

There has been no major break-through on the subject of leprosy control, but the importance of the diagnosis and treatment of early cases was emphasized; this can be done by mass surveys in hyperendemic areas and by contact tracing in other regions. Institutional isolation of infectious cases was not recommended. Views were given on the efficacy of BCG campaigns in the prevention of leprosy in hyperendemic areas, mostly (but not all) favourable.

A post-Congress symposium was held in Copenhagen, Denmark, on 20 and 21 August, enabling delegates to study the unique collection of skulls, bones and teeth at the Leprosy Museum, where are housed the best examples of the skeletons unearthed in Naestved between 1948 and 1968 from a burial ground in which over 600 leprosy sufferers were buried between the years 1250 and 1550. In addition, papers were read on leprous changes in the skull and teeth, followed by a lively discussion.

Bergen was chosen as the centre for the Congress as this is the city in which Dr Armauer Hansen discovered the leprosy bacillus 100 years ago, and our thanks and congratulations are due to the organizers for the excellence of all aspects of this memorable and well attended Congress.

W.H.Jopling

University College Hospital,
Hospital for Tropical Diseases,
4 St Pancras Way,
London, N.W.1

British Journal of Dermatology (1973) 89, 647.

Correspondence

THE EFFECT OF INTRACUTANEOUS GLUCOCORTICOIDS ON PLASMA CORTISOL LEVELS

SIR, In their article on the effects of glucocorticoids on plasma cortisol levels, Dr Zaynoun and Dr Salti compared betamethasone disodium phosphate/betamethasone acetate with triamcinolone acetonide. While their results are of interest, I do not believe they are entirely valid. Celestone Soluspan contains a total of 6 mg of betamethasone per ml, which is equivalent to triamcinolone acetonide 40 mg/ml. Unfortunately, the entire study compared full strength Celestone Soluspan with only one-quarter strength triamcinolone acetonide (10 mg/ml). If the authors truly wish to make a valid comparison, they should have diluted the betamethasone 1 to 4 to make it equivalent to the one-quarter strength triamcinolone. Incidentally, clinical observations have shown dilutions of Celestone Soluspan from 1 to 4 all the way down to 1 to 10 to be clinically effective. Several studies have been published that have shown that, when full strength triamcinolone acetonide 40 mg/ml has been injected, cortisol levels became depressed for up to 34 days (Hebley *et al.*, 1964; Arnold, 1968; Mikhail *et al.*, 1969, 1973; Editorial, 1969; Frey & Norman, 1970). As can be seen, this is considerably longer than the 10–13 days of suppression following betamethasone injection. Even smaller doses of triamcinolone acetonide have been shown to suppress for a far longer period than reported in this paper. It is interesting to compare the differences, and I wonder what caused the lack of suppression in this study.

Schering Corporation
Kenilworth
New Jersey 07033, U.S.A.

GERALD N. WACHS

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SIR, We thank Dr Wachs for his remarks and criticisms. They give us the opportunity to emphasize some of the points mentioned in our article. We are aware of the fact that Kenacort-A and Celeston Soluspan injections do not have equivalent concentrations of the steroids per ml, and that 0·75 mg of betamethasone is equivalent to 4 mg of triamcinolone. Our concern was to compare the effects on plasma cortisol of different amounts of the two preparations when injected into normal healthy skin. One ml of Celeston Soluspan, which is equivalent to 32 mg of triamcinolone acetonide, caused a more marked suppression of plasma cortisol than 50 mg of triamcinolone acetonide, when injected intracutaneously. The difference becomes more apparent if we consider only the long acting component of the Celeston injections, i.e. the betamethasone acetate 3 mg/ml, since this remains in the skin after the short acting component (betamethasone disodium phosphate) disappears. In addition, the administration of 5 ml of Kenacort-A (10 mg/ml) intracutaneously involved a bigger area than that needed for 1 ml of Celestone Soluspan. This could imply more absorption of the former drug. Hence, the use of 40 mg/ml could make the difference between the two different drugs even greater. The investigations of Porter *et al.* were done on patients with cystic acne in which the absorption of the injected drug is expected to be greater than in the normal skin. Moreover, even with the use of 50 mg of triamcinolone acetonide (40 mg/ml) there was a suppression of plasma 11-hydroxy corticosteroids for 5 days only. McGugan *et al.* have found that a single injection of 75–100 mg of full strength triamcinolone acetonide tertiary-butyl acetate (40 mg/ml) induced adrenal suppression which persisted for 4 days in most subjects. By contrast, with 25 mg of triamcinolone diacetate or 50 mg of triamcinolone acetonide tertiary-butyl acetate the suppression was occasional and transient. Our results in the group of patients who received the 50 mg triamcinolone acetonide injection were similar. In none of the concentrations used was the adrenal suppression as prolonged as with the group who received the Celestone Soluspan 5 ml dose. The delayed suppression in the latter group lasted up to the thirteenth day. It is important to note that 5 ml of Celestone Soluspan are equivalent to 80 mg of triamcinolone acetonide, if we consider the long acting component alone, as the short acting component is not responsible for the delayed effects of the Celestone Soluspan injections.

Divisions of Dermatology and Endocrinology
Department of Internal Medicine
American University of Beirut

SHUKRALLAH T. ZAYNOUN
IBRAHIM S. SALTI

EARLY FEATURES OF THE STRAWBERRY MARK

SIR, I found the article by Dr Hidano and Dr Nakajima on early features of the strawberry mark most illuminating.

For a period in 1971–72, I made routine examinations of the newborn under the care of Dr Cyril Kesson in St George's Hospital, London; the average birth rate at that time was eighty-four per month. In November 1971, I noticed an unusual telangiectasis which radiated from its centre but was not a spider naevus; this was separated from normal skin by a clearly defined halo of pallor. I was not able to find this lesion described as my search of the literature did not include Payne's article in the *Journal of Plastic and Reconstructive Surgery*. I subsequently found four more examples, giving an incidence of just under one per 100 babies. All were on the thigh or trunk and varied from 1 to 3·5 cm in diameter. One subsequently developed into a strawberry mark 'en plaque'. Later, at another hospital, I found the incidence approximately the same.

The lesion can easily be missed unless any area of telangiectasis or pallor is rubbed firmly with the finger, when the vasodilatation of the surrounding skin and the pallor of the halo are markedly contrasted. This also occurs when the baby is crying vigorously or has just had a warm bath.

I wish to raise three further points:

(1) There are clinical differences between the classical strawberry mark and the strawberry mark en plaque; are there basic pathological differences?

(2) Could somebody propose a more euphonious name than 'abortive angioma' or 'halo telangiectasis'?

(3) Although the presence of a halo telangiectasis gives warning that a strawberry mark may appear, I believe that in most European babies the lesions disappear without further progression.

Charing Cross Hospital
Fulham Palace Road
London W6 8RF

RICHARD G. WILSON

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THE TREATMENT OF CAVERNOUS HAEMANGIOMA WITH TOPICAL BETAMETHASONE 17 VALERATE

SIR, Systemic corticosteroids have been successfully employed in the treatment of large cavernous haemangiomata, but such treatment involves obvious hazards. In an attempt to secure more rapid involution than could be expected to occur spontaneously, but at less risk to the patient, we have used topical betamethasone. Figs. 1–7 and their legends tell the story. Only a large series of cases with matched controls will establish its possible significance.

G. WEBER

Skin Clinic
City Hospital
85 Nurnberg 5
Germany

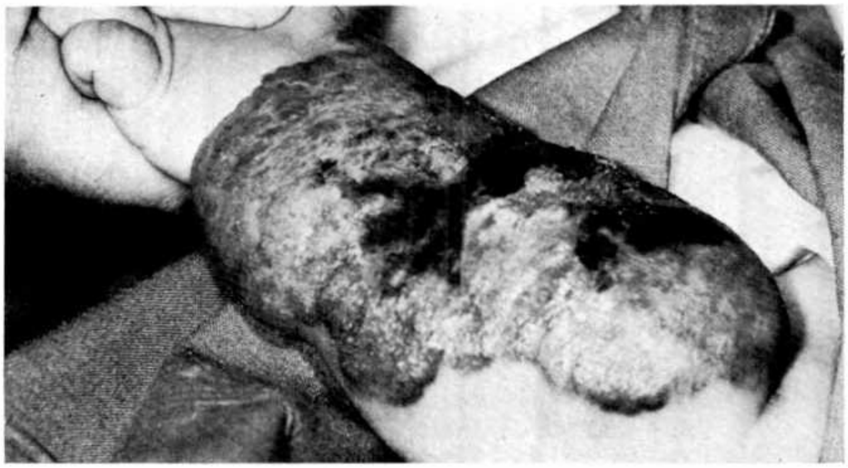

FIGURE 1. Girl aged 6 months. Cavernous haemangioma continuing to enlarge since birth (October 1969).

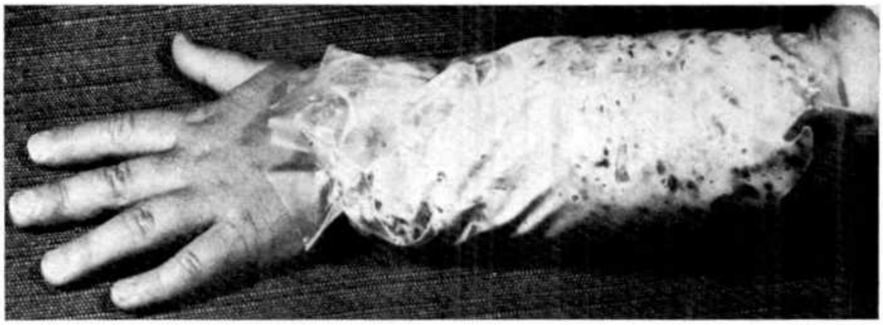

FIGURE 2. Application of betamethasone valerate cream under occlusion for 6 h daily (October 1969).

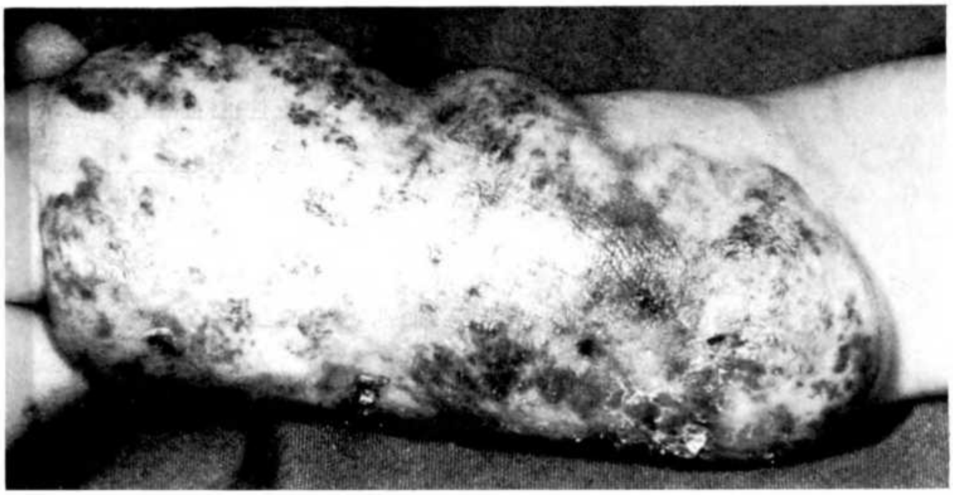

FIGURE 3. Regression under continuing treatment (January 1970).

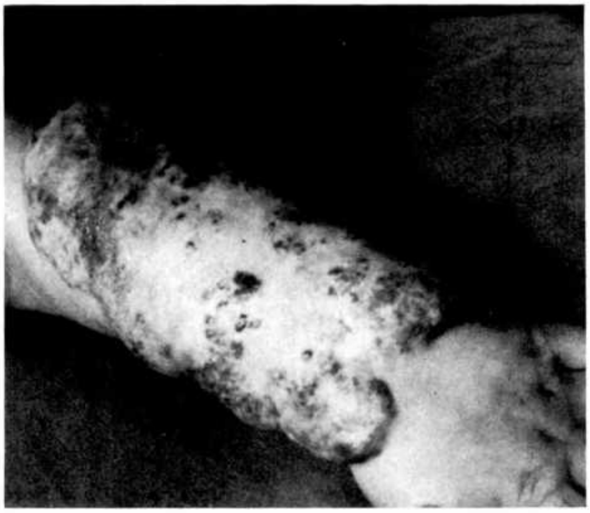

FIGURE 4. Regression under continuing treatment (April 1970).

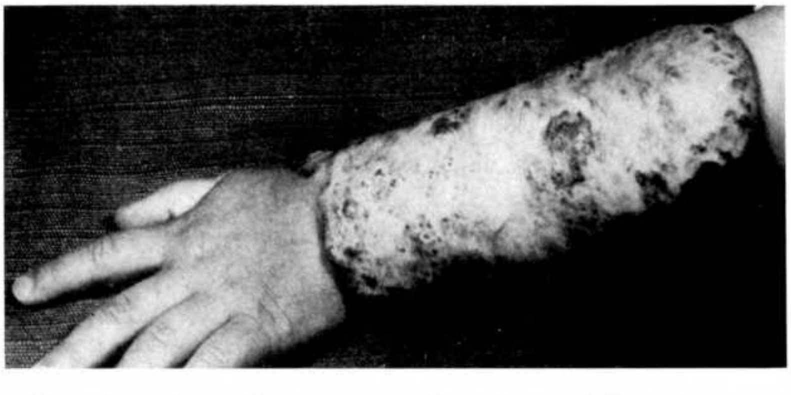

FIGURE 5. Regression under continuing treatment (September 1970). From September 1970 the cream was applied without occlusion.

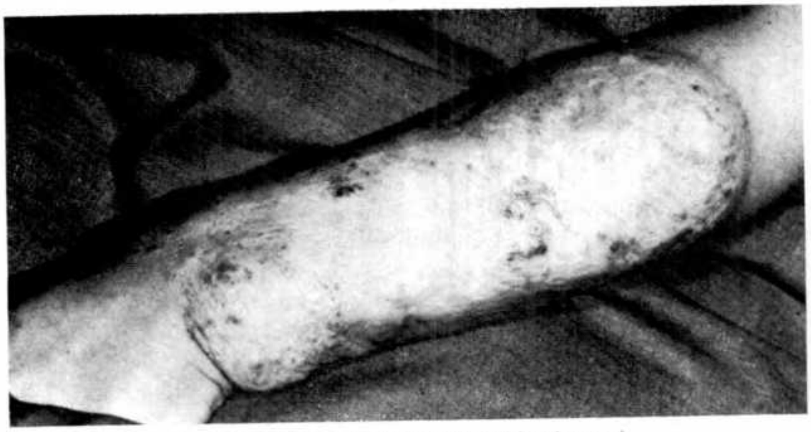

FIGURE 6. Continued regression (March 1972).

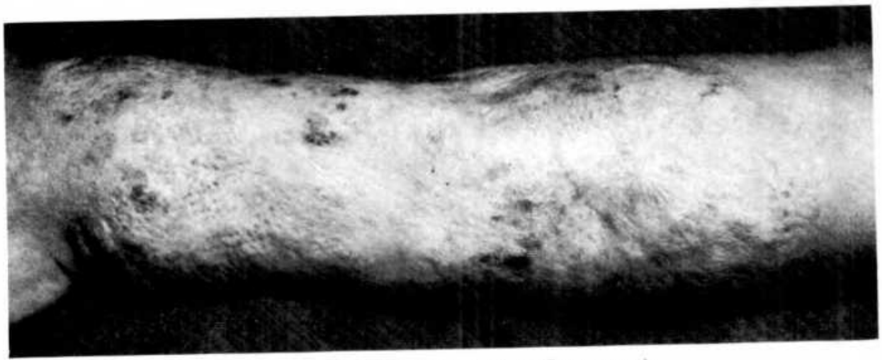

FIGURE 7. Continued regression (June 1973).

PSEUDOSARCOMATOUS DERMATOFIBROMA

SIR, I would like to add a note of caution concerning publications about the 'pseudosarcomatous' group of tumours (Thavabalan & Ratnaike, 1973), supposedly benign. I have personal experience with two published cases in which death was caused by metastatic tumour (Schreiber, 1970; Yaffee, 1970).

We are beginning to realise that the interaction of host with tumour is a complex process of which morphology is only a crude index of what is to come. For this reason I prefer the term 'masked sarcoma.' What is needed is a reliable biochemical or immunological marker of prognostic significance. Until then all physicians should regard the pathological diagnosis of 'pseudosarcoma' with great caution.

HOWARD S. YAFFEE

Department of Dermatology
Boston University School
of Medicine
Boston, Massachusetts
U.S.A.

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Book Reviews

Dermatologie und Venerologie. Eine Propadeutik und Systematik. A.GREITHER (1972) Berlin, Heidelberg, New York: Springer. Pp. xiv + 212, 82 illustrations. Price £2.83.

No two experts agree on what an ideal textbook of dermatology for undergraduates should offer. Professor Greither of Düsseldorf sees as one of the main temptations in this visually oriented subject, that authors tend to rely more and more on illustrations, whereas no amount of pictures can replace the thought processes and the understanding essential in medical education. Similarly, he points out that whilst some of the more modern question and answer techniques may teach the student to become good at guessing answers, they will not help him to understand fundamental processes. He, therefore, goes on to offer single-handed a compact, clear and well arranged text without a single photograph but with numerous schematic drawings, and concentrates on analysing, interpreting and understanding basic concepts rather than expecting the student to memorize facts and pictures.

With absolute thoroughness, he covers it all; histological and clinical fundamentals, venereology, infections, adnexal diseases, tumours, pigmentary and other diseases. The headings are clear, the subheadings well arranged, the material is almost consistently reliable; the drawings are pleasant and easy to follow; and the type, the paper, and the price are all very reasonable.

I cannot fault this book in its aims, outlook or presentation, and yet I will not give it the coveted prize of 'Ideal textbook of dermatology for undergraduates'. The missing factor is inspiration. Perhaps a little wit or humour, or something unexpected here and there, would have made all the difference. The quality is there; the spark is missing.

I.SARKANY

Essays on Tropical Dermatology. JAMES MARSHALL (Ed.) (1972) Volume 2. Amsterdam: Excerpta Medica. Pp. xii + 459, illustrated. Price £14.46.

The late Robert Simons planned the first volume of 'Essays on Tropical Dermatology', published in 1969, as a supplement to his 'Handbook of Tropical Dermatology'. James Marshall took over the editorship of Volume 1 of the Essays, after the sudden death of Dr Simons, and has sole responsibility for this second volume. In his preface he refers to the vague boundaries of 'tropical dermatology' and he has clearly chosen his contributors with the intention of dispelling the persistent illusion that 'tropical dermatology' is concerned largely with exotic diseases. As Marshall says, any book on tropical dermatology could be subtitled 'the dermatoses of the poor'. In a brief but effective essay, Sharat Desai points out that scabies, pyodermas and dermatophytoses account for over 40% of dermatological out-patient attendances at his hospital in Bombay. Similar or higher figures for the common infectious diseases were regularly reported in parts of Britain less than 50 years ago. A chapter by Wajdi Kanan shows how the discovery of oil, and the establishment of a free health service and a higher standard of living, help to bring such infections under control.

The thirty-nine essays in this book cover a very wide range. The editor himself writes on 'The White Skin and the Sun', Degos and his colleagues on 'Actino-reticulosis' (better known as actinic reticuloid), Burry on 'Photocontact Dermatitis', and Malherbe on the 'Surgical Treatment of Skin Tumours of White People'. Also concerned with climatic factors is a useful review of miliaria by Leeming. Canizares, writing on the dermatoses of Latin America, emphasizes the influence of altitude on climate and refers to chilblains in tropical latitudes.

Three essays remind us of the need for more studies on racial factors in disease incidence. Scott gives a well illustrated account of 'Skin Diseases in the South African Bantu', Basset and his colleagues discuss their personal experiences of 'collagenoses' in West Africa, and Rippey and Schmaman write on 'Skin Tumours of Africans'. All these are useful contributions. It has become evident that many earlier investigators not only made unwarrantable generalizations on inadequate evidence, but wrongly assumed that each of such vast racial groups as the Negroids, Caucasoids or Mongoloids was genetically more homogeneous than the physical anthropologists knew it to be; for example there are significant non-environmental differences in disease incidence between West Africans and East Africans.

Climate and custom, however, are probably more important than race in inducing Mudi-chood, a new dermatosis, described by Sugathan and Nair of Kerala State.

Many of the remaining chapters are concerned with traditional 'tropical dermatology', cordylobiasis, onchocerciasis, leprosy and phycomycosis, but the editor has included an essay on scabies by Zumpt and a welcome and stimulating defence by Gear of the patho-genetic propensities of Demodex folliculorum.

This is an excellent book. It can be read with profit and pleasure by dermatologists wherever they work, not only because their widely travelled patients may confront them any day with yaws or Lobo's mycosis, but because many disease mechanisms are of universal application and because greater insight into the ecology of skin disease is as much a necessity in Lambeth as in Lagos. The volume is well designed, well produced and well edited—but why did the editor allow the author of the very useful essay on 'Virus Infections in Africa' to omit all but one of his references? Other essays are well documented.

ARTHUR ROOK

The Phakomatoses. P.J.VINKEN AND G.W.BRUYN (Eds.) (1972) Amsterdam: North Holland Publishing Company. Pp. xi+821, 373 illustrations. Price £36.54.

This is Volume 14 of the 'Handbook of Clinical Neurology', which will eventually consist of thirty volumes. The Editorial Advisory Board responsible for the Handbook as a whole is international in composition, and the editors of the present volume have drawn on most European countries and on Canada and the United States, to assemble their team of authorities. All the chapters are in English, the clarity of which, with rare exceptions, is a tribute to the translators and editors.

In their foreword the editors admit that the phakomatoses are perhaps a heterogeneous group of disorders, and they speculate on the reason for the relative lack of interest shown in them by the English-speaking world. The fact that such has been the case justifies, if justification be needed, the preparation of an exhaustive treatise in English on these rare disorders.

A chapter has been devoted to each of the more important phakomatoses and to some of the less well known, and provides a detailed and well illustrated review of all aspects of the syndrome, with a really comprehensive bibliography. Other chapters consider the phakomatoses as a whole, concentrating on their differential diagnosis (Liebaldt and Leiber), genetics (Koch), cutaneous lesions (Musger), and on other aspects. A final chapter is concerned for good measure with miscellaneous neurocutaneous diseases.

It is difficult to find fault with this book. Heterogeneous though these disorders may be, the editors have so tied the material together that it forms a more or less homogeneous entity. No work on science, or indeed on history for that matter, can ever be 'definitive', but for many years to come this book will be the standard reference on the phakomatoses; and it should not be used only for reference. In reading the detailed accounts of rare but familiar syndromes, most dermatologists will find much that is illuminating and will increase their understanding of the mechanisms underlying lesions seemingly so disparate that their significant association can be recalled only with the help of a mnemonic.

ARTHUR ROOK

Holzarten mit Gesundheitsschädigenden Inhaltsstoffen. BJÖRN M.HAUSEN (1973) Stuttgart: DRW-Verlags-GmbH. Pp. 148. Price £2.60.

There can be few dermatologists of any experience who have not become painfully aware of the frustrations of investigating a case of contact dermatitis unquestionably caused by wood. The trade names of some timbers are applied to botanically (and immunochemically) unrelated timbers which happen to be similar in appearance or in certain physical properties. The same species is sometimes given different trade names in its various countries of origin and yet others in importing countries.

Dr Hausen has written a brief, clear text in which he systematically summarizes available knowledge of those timber species with constituents injurious to health. There are excellent cross references between trade-names and botanical names. There is no bibliography, but the decision not to include one was probably wise; had it been comprehensive it would have increased very greatly the cost of the book. As it is, it is a first-class inexpensive source of information indispensable to any dermatologist who investigates his patients with contact dermatitis.

ARTHUR ROOK

Mycetoma. E.S.MAHGOUB AND I.G.MURRAY (1973) London: William Heinemann Medical Books. Pp. vii + 132, 52 illustrations, 4 colour pages. Price £3.50.

This is the first book in English on mycetoma since H. V. Carter's monograph *On Mycetoma or the Fungus Disease of India*, published in 1874. Our knowledge of the disease since that date has advanced in all aspects except one—therapy. Most mycetomas are still treated by surgery and the recurrence rate, even with radical surgery, is high.

The authors prefer the term *maduromycosis* to describe mycetoma caused by a true fungus, and *actinomycosis* to describe one caused by an actinomycete. The distinction is important, for actinomycetoma may be treated with sulphonamide or sulphone drugs, such as dapsone, with complete cure in a useful proportion of cases; maduromycetoma can rarely be so treated, even with antifungal drugs, and radical surgery remains the treatment of choice. Although the mycology of this disease is not unduly complex, it may prove difficult for a clinician remote from a health centre to preserve biopsy material free from contaminant fungi, which may complicate accurate diagnosis. It is fortunate, therefore, that quite accurate diagnosis of the infecting organism may be made by a study of the grains in tissue preserved and sectioned for histopathology.

Whilst the disease occurs principally in tropical countries, with Africa and India as the areas of greatest prevalence, South America, the southern United States and especially Mexico contribute a large proportion of cases; fewer cases are reported from Asia. Cases are reported from eastern Europe; Bulgaria and Rumania contributed fourteen of 854 cases in a world-wide survey published in 1963.

The book is well illustrated by black and white photographs; the colour plates, which are of fungi in tissue and in culture, are probably not detailed enough for diagnostic use as there are six or eight to a page. Omission of these plates would not have detracted from the value of this book and would presumably have made it even less costly. The appendices draw together methods for the isolation and identification of the causative organisms. Eleven fungi and six actinomycetes may be involved.

This is an interesting book and a fitting tribute to the late Ian Murray. It will be read with profit by all and will prove invaluable to those likely to receive patients for diagnosis or treatment.

W.C.NOBLE

These and Dissertations

Anti-Leprosy Prophylaxis in Countries of Low Endemicity: BCG in Leprosy Prophylaxis. Thesis presented by D.BENIGNO PEREZ PEREZ, Salamanca University, Spain, 1972.

This study deals with the Mitsuda reaction (lepromin test) as an index of resistance to leprosy, the conversion of previously negative Mitsuda tests in healthy people by vaccination with BCG, and the value of such conversion as an index of resistance to leprosy.

The value of the Mitsuda reaction is once again confirmed. BCG vaccination of negative Mitsuda reactors is shown to be of considerable prophylactic value, though the protective action of BCG has yet to be established conclusively.

If a negative Mitsuda reaction does not convert to positive after one BCG vaccination, repeated vaccination may produce conversion. It is also stated that when BCG is given, in all except anergic cases, there is a reduced incidence of leprosy, and the course of the disease is more benign.

W.H.Jopling

British Journal of Dermatology (1973) 89, 657.

Quarterly Review

CONTACT DERMATITIS XXIV

The Wide World of Antimycotics

The management of fungous infections of the skin is part of the everyday work of the dermatological practitioner. Mycosis of the foot, the so-called athlete's foot, is regarded as an epidemic disease of modern times (Götz, 1962, 1966). One of the reasons for the steady increase of this condition is that we prefer closed shoes and synthetic stockings, whereas formerly sandals or even bare feet were favoured (Nishio, 1966).

With the rising number of patients suffering from fungous infections, medicaments came on the market to facilitate their treatment. The earlier preparations contained mainly rosaniline dyes, resorcinol, sulphur, salicylic acid and formaldehyde. Contact allergies to these substances are rare; only formaldehyde, and to some extent resorcinol, contribute to the problem of dermatitis from applied medicaments (Bandmann *et al.*, 1972; Epstein & Maibach, 1966; Keil, 1962; Sturde & Reichenberger, 1965). The systemic treatment of mycoses with griseofulvin is without adverse side effects. This peaceful picture of allergic innocence was completely destroyed by modern drugs for external use. Here we come across some potent allergens and even photo-allergens. Unfortunately, group reactions to substances incorporated in cosmetic products and those in occupational use are common; the allergic potential of antimycotics is therefore of great importance.

It is practically impossible to cover in a short survey the whole field of contact allergies against substances utilized as antimycotics; a *tour d'horizon* will serve to highlight the situation.

To begin with, contact allergies to hydroxyquinolones used to be rather rare events (Bandmann, 1966; Pankok, 1964). Recent publications (Bandmann *et al.* 1972; Breit, 1972) report a much higher number of allergies. Iodochlorhydroxyquinolone (Vioform) contributes 3·1% to the high rate of sensitization in patients suffering from stasis dermatitis and ulcers of the lower leg (Breit, 1972). One percent of 4000 patients showed a positive reaction to chlorquinaldol (Sterosan) (Bandmann *et al.*, 1972), which is used virtually exclusively as an antimycotic agent. The hydroxyquinolines are the second classic example of group reactions after the so-called para-group (Bandmann, 1970; Bandmann & Dohn, 1967; Bandmann & Fregert, 1973). Chemicals of this order are incorporated in antimicrobic preparations for medical and industrial usage. The rate of sensitization seems to depend on the type of vehicle in which these substances reach the skin, because allergies are more common in countries in which hydroxyquinolines are usually prescribed in ointments or creams and not in pastes or suspensions (Bandmann & Fregert, 1973). It should be kept in mind that hydroxyquinolines are often added to corticosteroid creams to prevent bacterial growth.

In both organic and inorganic forms, mercury preparations are potent sensitizers (Wilkinson, 1972). Particularly, phenylmercuric borate in a concentration of 0·066% in an alcoholic solution is widely used for treating athlete's foot and for preparing the skin for surgical procedures. It is not at all surprising, therefore, that 116 of 1094 contact allergies to applied medicaments were to this substance (Bandmann, 1966). An interesting fact is that many of these patients cross-react to other mercuric compounds, which can be found as preservatives in creams and ointments, but are often not declared on the label (Bandmann, 1970).

The problems of photocontact allergy concern three antimycotic medicaments, bis(5-chloro-2-hydroxyphenyl)sulphide (Fenticlor) and 5-bromo-4′-chlorosalicylanilide (Multifungin), which are chemically related to the halogenated salicylanilides, and 4-chloro-2-hydroxybenzoic acid-N-*n*-butylamide (Jadit), which belongs to the group of sulphanilamides (Burry, 1967, 1968; Burry & Hunter, 1970; Ippen, 1961; Tronnier, Turek & Janns, 1969). Although the risk of sensitization is not very high, the first mentioned substances in particular should not be applied to light-exposed areas, because there is the risk of the patient becoming a so-called persistent light reactor, an incapacitating state which necessitates avoidance of all sunlight (Burry, 1967; Epstein, Wuepper & Maibach, 1968). In addition, many soaps and cosmetic products contain halogenated salicylanilides or related compounds. The avoidance of contact is not easy because knowledge of their composition cannot be obtained by the average consumer.

Focusing on tetramethylthiuram disulphide, we have a substance which is used in medicine and in several industrial processes. It serves as an antifungous medicament, preserves wood and technical oils and disinfects fruits; nevertheless, its main use is by the rubber industry as one of the most important chemical additives (Bandmann & Fregert, 1973). So reports of contact allergies to this substance originate from many different quarters (Bandmann & Nasemann, 1958; Brehm, 1968; Schulz & Herrmann, 1958; Wilson, 1969). The incidence of sensitivity to tetramethylthiuram disulphide was 2% in the joint European study published by the ICDRG (Fregert *et al.*, 1969).

Many other substances used mainly in treating fungous infections of the feet are incriminated as sensitizers (Pankok, 1964; Schön, 1969). A suggested test series was recommended by Bandmann & Dohn (1967).

Although the possible allergens in the group of antimycotic remedies are numerous, the problems can be reduced to a few straightforward statements. The existence of a fungous disease should be verified by culture in each case (Götz, 1962). One should keep in mind that the classic antimycotic substances are still effective. If we employ modern preparations we often pay for the greater comfort of usage with a higher risk of sensitization, because fungous infection and inflammation predispose the skin to develop contact allergy (Schulz, 1966). In many instances an apparently unsuccessful treatment of mycosis does not imply that the organism has become resistant, but raises the suspicion of dermatitis from the applied medicament. Patch testing can reveal the allergen. Sometimes the use of purely antifungous agents can lead to an overgrowth of Gram-negative organisms and to clinical exacerbation, as recently stated by Leyden & Marples (1973). Of course, other dermatoses, for instance psoriasis or atopic dermatitis, can mimic a fungous disease too. In any case of an interdigital dermatitis of the toe web only a careful investigation which includes patch testing will satisfy both the patient and his doctor.

REINHARD BREIT
HANS-JÜRGEN BANDMANN

Dermatologische Klinik und Poliklinik der Universität,
D-8000 München 2,
Frauenlobstraße 9,
West Germany

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News and Notices

International Symposium on Wound Healing

The International Symposium on Wound Healing will be held at Rotterdam, The Netherlands, from 8 to 12 April 1974. Further information about the Symposium may be obtained from the Secretariat, c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague, The Netherlands.

First Hair Transplant Symposium and Workshop

The American Society for Dermatologic Surgery and The American Academy of Facial, Plastic and Reconstructive Surgery, Inc., are co-sponsoring this conference which is designed to offer an opportunity for the exchange of ideas among various disciplines, and to present the latest advances in techniques of hair transplantation. It will be held on 8 and 9 February 1974, at the Stough Dermatology and Cutaneous Surgery Clinic, P.A., Doctors Park, Hot Springs, Arkansas 71901, U.S.A. Attendance will be limited. The speakers will include dermatologists, otolaryngologists and plastic surgeons. For further information, contact D.B. Stough, III, M.D., Program Director (address as above).

European Society for Dermatological Research

The ESDR meeting in 1974 will be held in Amsterdam on 23 and 24 April. Attendance at the scientific sessions will be open to all members, contributors introduced by members, and guests. Non-members who wish to present a paper are requested to send an abstract to the Secretary. They will be invited to attend the meeting as contributors or as guests. Forms for abstracts are available from the Secretary, Mons. Prunieras, Fondation Ad. de Rothschild, 29 rue Manin 75019, Paris, and must be received by 15 December. Applications for membership should also be addressed to him.

British Association of Dermatologists Summer Meeting and the Anglo-French Reunion 1974

The Annual Meeting of the British Association of Dermatologists will take place in London on the 3, 4, 5 and 6 July 1974 under the Presidency of Dr Hugh Wallace. The Meeting will be combined with an Anglo-French Reunion, the Anglo-French day being the 5 July. Papers are invited and should be sent by the 1 March 1974 in the form of a summary in triplicate of approximately 200 words, to the Honorary Secretary, Dr J.S. Pegum, The London Hospital, Whitechapel Road, London E1 1BB. The *British Journal of Dermatology* hopes to publish in a Supplement the summaries of those papers which are accepted. These should therefore be detailed, precise and explicit and avoid such phrases as 'the results will be discussed'. Any references should be set out as required by the *British Journal of Dermatology*.

Postgraduate Fellowship in Clinical Dermatopharmacology

Applications are being accepted for a fellowship in Clinical Dermatopharmacology jointly sponsored by the University of California, San Francisco, and Syntex Laboratories. It covers a 1-year period starting 1 July 1974. The salary is $14,000 per year. Those interested please send their *curriculum vitae* to William L. Epstein, M.D., Acting Chairman, Department of Dermatology, University of California, San Francisco, California 94143, U.S.A., as soon as possible.

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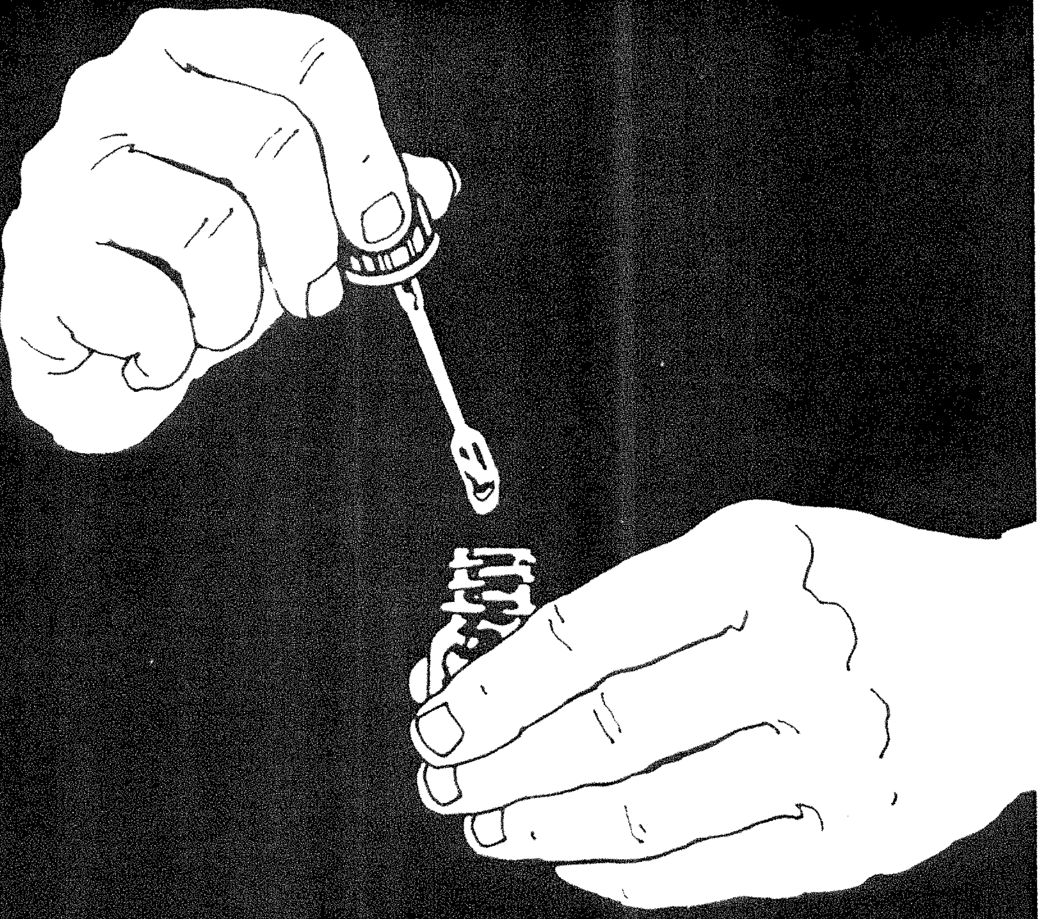

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(Experimental Investigations on the Differentiation of the Carcinomas of Skin)

Zala, L. (Bern): Satellitenförmige Rezidive bei teleangiektatischem Granulom
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milligram(s) (10^{-3} g)	mg	second(s)	s	gravitational acceleration	g
microgram(s) (10^{-6} g)	μg	cubic millimetre(s)	mm^3	micrometer(s)	μm
nanogram(s) (10^{-9} g)	ng	millimetre(s)	mm	percent	%
picogram(s) (10^{-12} g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
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FOREWORD

Since the first meeting of the British Association of Dermatology in 1921, under the presidency of Sir Malcolm Morris of St Mary's Hospital, London, a summer meeting has been held each year. Many of the papers presented to the Association on these occasions have later been published in this journal. However, the clinical meetings which form an important part of the proceedings have never been permanently recorded. The Executive Committee of the Association felt that this omission was regrettable, since cases of special interest and importance are regularly demonstrated. The generosity of Messrs Glaxo, which is here gratefully acknowledged, has made it possible to publish as a special supplement reports of the cases and summaries of the papers to be presented to the Association in Cardiff in July 1973. This supplement will be distributed to all subscribers to the journal.

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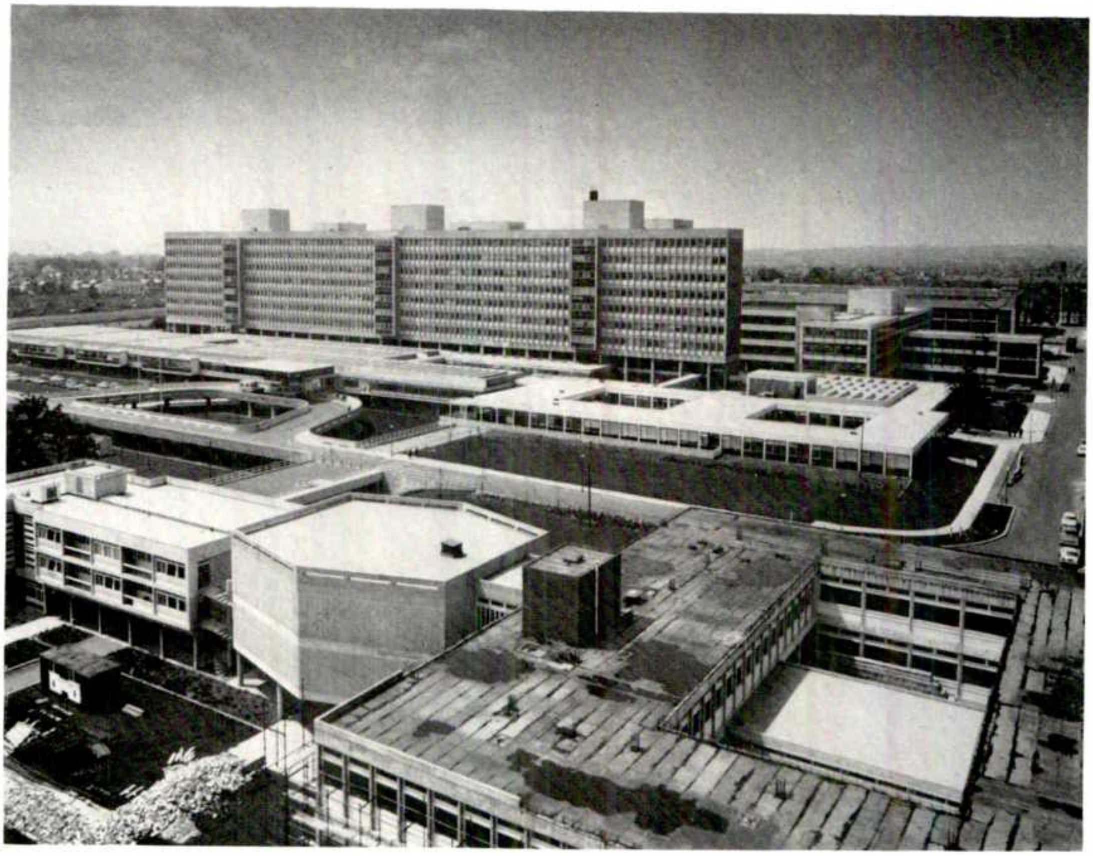

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British Journal of Dermatology (1973) **89**, Supplement 9, 4.

British Association of Dermatology Fifty-third Annual Meeting, 1973

PRESIDENT: DR G.A. HODGSON

THURSDAY–SATURDAY, 12–14 JULY 1973
LECTURE THEATRE 1, THE MEDICAL SCHOOL,
UNIVERSITY HOSPITAL OF WALES

PROGRAMME OF MEETING

Thursday 12 July

8.30	Registration	
9.15	Annual General Meeting (Members only)	
10.15	**Coffee**	
10.30	Official photograph	
11.00	Geoffrey Hodgson	Wales, its history and dermatology
11.20	J. Picton Thomas	Androgen metabolism in the skin of hirsute women
11.40	C. F. H. Vickers	Iron deficiency and the skin
12.00	Ronald Church	Methyl dopa causing a seborrhoeic dermatitis-like eruption
12.20	N. R. Rowell	A painful bleeding syndrome associated with increased fibrinolytic activity
12.40	**Lunch**	
14.00	S. Jablonska and T. Chorzelski	Transitional forms between dermatitis herpetiformis and bullous pemphigoid

14.20	T. M. Phillips, J. Everall and D. Viza	Pemphigus and pemphigoid serum patterns in malignant tumours, benign tumours and normal skin
14.40	P. P. Seah, M. R. Mazaheri and Lionel Fry	Complement in the skin of patients with dermatitis herpetiformis
15.00	Parveen J. Kumar, D. B. A. Silk, R. Marks, M. L. Clark and A. M. Dawson	Functional and morphological changes in response to a gluten-free diet and corticosteroids in dermatitis herpetiformis and adult coeliac disease
15.20	M. Lancaster-Smith, Parveen, J. Kumar, R. Marks, M. L. Clark and A. M. Dawson	Immunofluorescence of the jejunal mucosa in dermatitis herpetiformis and adult coeliac disease
15.40	**Tea**	
16.10	R. K. Winkelmann, G. J. Gleich, S. A. Muller and R. E. Jordon	IgE and diseases of the skin
16.30	John H. S. Pettit	Skins in the East
18.30	President's Reception at The National Museum of Wales, Cathays' Park, Cardiff	

Friday 13 July

8.30	Registration	
9.00	C. A. Ramsay and A. V. J. Challoner	Changes in cutaneous blood flow after ultraviolet radiation at 250 nm and 300 nm
9.20	T. Laksmipathi, Joyce Herd, J. Rogers and W. Frain-Bell	Chronic dermatitis with photosensitivity and its relationship to actinic reticuloid
9.40	B. E. Johnson	Chlorpromazine phototoxicity: a non-classical mode of action
10.00	P. E. Hutchinson and R. S. Wells	Woolly hair
10.20	I. W. Whimster	Preliminary observations of an immunologically determined dermatosis in reptilian skin homografts
10.40	**Coffee**	

11.10	A. S. Breathnach	Freeze-fracture replication of melanocytes and melanosomes
11.30	E. Abell, R. Marks and E. Wilson Jones	Secondary syphilis: a clinico-pathological reappraisal
11.50	R. Marks and A. Griffiths	The epidermis in pityriasis rubra pilaris: a comparison with psoriasis
12.10	E. Abell, T. Nishikawa and R. Marks	The regenerative capacity of the basement membrane region
12.30	**Lunch**	
14.00	Coaches leave the University Hospital for the visit to the Welsh Folk Museum, St Fagan's Castle	
17.00 (approx.)	Coaches leave St Fagan's for the Angel Hotel and University Hall	
The Dowling–McCaw Golf Competition	After lunch at the Hospital, the competition will be held at the Newport Golf Club, Rogerstone	
The Bowers–Sneddon Sailing Trophy	After lunch at the Hospital, the competition will be held at the Llanishen Reservoir	
19.30 for 20.00	The Annual Dinner at the Angel Hotel	

Saturday 14 July Clinical meeting

9.30	Cases in the Out-patient Department of the hospital
10.30	**Coffee** in the foyer of Lecture Theatre No. 1.
11.00	Discussion of cases in Lecture Theatre No. 1
12.30	**Sherry and Lunch** at the Lakeside Restaurant as guests of the Management Committee of the University Hospital of Wales

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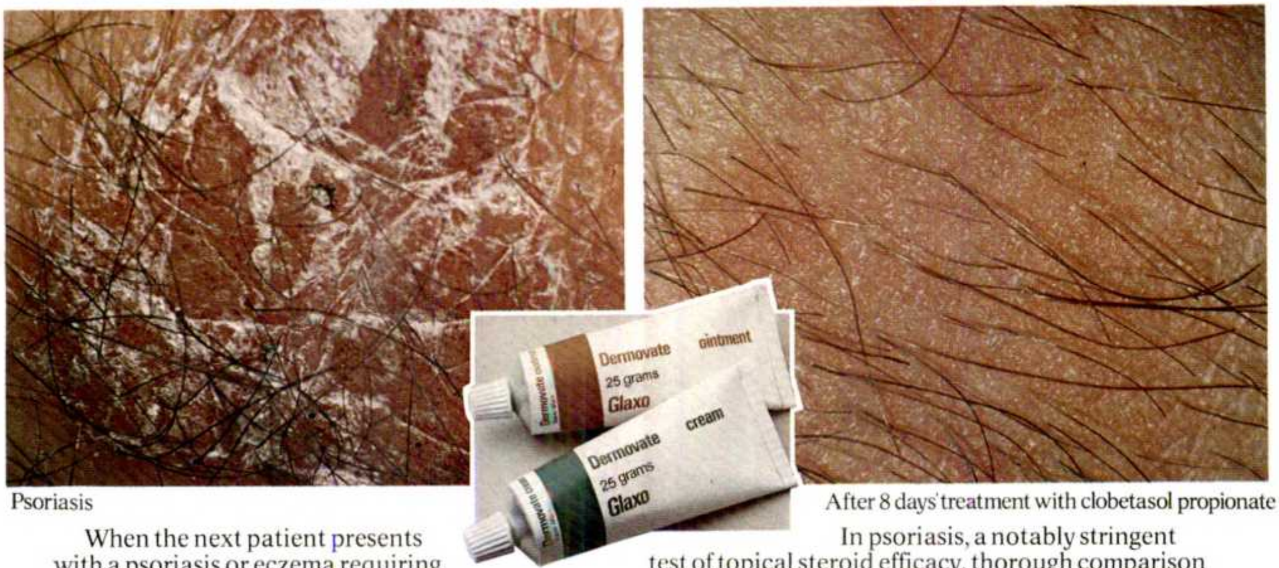

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British Journal of Dermatology (1973) 89, Supplement 9, 9.

SUMMARIES OF PAPERS

Wales, its history and dermatology

GEOFFREY HODGSON

University Hospital of Wales, Cardiff

The pathway to Welsh dermatology of today is traced from the days of primitive healing, associated with religion and magic, in ancient 'Cymru' against the background of a short history of Wales, which was made turbulent by the many invaders affecting her culture and education within her mountains.

Some dermatology was practised by Celtic druids, harassed by Roman legionaries, and by the clergy of the Christian church, before the Anglo-Saxon leeches and the Court Physicians (e.g. 'Physicians of Myddfai') of the early great Welsh kings, recorded their cures in ancient manuscripts. It was later practised by post-Reformation graduates, trained in English or other universities, educated gentry, simple village herbalists or itinerant quacks of mediaeval times. Later the country doctor of modern undeveloped Wales and the miners' doctor of industrialized Wales preceded the hospital dermatologist. Linking all these is the traditional use of holy wells, herbal and animal ointments, magic and superstition; relics of the most ancient religious and healing rites, persisting sometimes to modern times.

Androgen metabolism in the skin of hirsute women

J. PICTON THOMAS

University Hospital of Wales, Cardiff

In vitro incubation of skin with labelled androgens has shown that the utilization and metabolism of dehydroepiandrosterone (DHA) in women with idiopathic hirsutism is similar to that seen in the normal male.

Women with idiopathic hirsutism may be distinguished from other hirsute woman by the long history and absence of other manifestations of virilism. Biochemically, such patients are known to produce an excess of testosterone, but this is readily suppressed with dexamethasone.

We have studied metabolism of labelled androgens in the skin of five hirsute women and found the utilization of DHA to be much higher than in normal women, but comparable to that of normal males. By contrast, utilization of DHA in a hairless, hypogonadal male was similar to that seen in normal women. Of the metabolites formed, the 17-ketosteroids predominated in all, but a higher proportion of 17-β-hydroxysteroids was found in hirsute women and in the normal male, agreeing with results of urinary steroid assays obtained by others.

Preliminary data would indicate that the ready conversion of DHA in the skin is but one manifestation of a more general disturbance in androgen metabolism, whereby the adrenals and ovaries secrete an excess of testosterone. The fact that testosterone utilization was similar in all subjects would imply that the high production rate did not induce excessive activity of those enzymes concerned with androgen metabolism by the skin.

Iron deficiency and the skin

C.F.H.VICKERS

Liverpool Royal Infirmary

The results of a 14-year survey of the relationship between iron deficiency, generalized pruritus and alopecia will be presented.

A series of seventy-six patients with generalized pruritus and no obvious skin disease disclosed fifty-nine with abnormal serum iron levels. Although only fifteen were anaemic, in males such results were found to be ominous, as twelve out of thirteen were eventually found to have underlying neoplasia or reticulosis during the follow-up period. A clear relationship between the pruritus and serum iron levels was established in several of the patients.

Thirty-one patients with telogen effluvium were observed. The only cause appeared to be iron deficiency, and the hair counts bore a clear relationship to the serum iron levels.

Methyldopa causing a seborrhoeic dermatitis-like eruption

RONALD CHURCH

Royal Infirmary, Sheffield

Eczematous eruptions due to systemically administered drugs are uncommon, well authenticated causes being aminosalicylic acid and dihydrostreptomycin (Crissey, Osborne & Jordan, 1957).

Within the past 3 years, thirteen patients have been seen suffering from an eruption resembling seborrhoeic dermatitis while taking methyldopa for the control of hypertension. They consisted of four males and nine females in the age range 45–79 years. Some had taken the drug for many months before the eruption commenced, in others the rash developed within weeks or after an increase in dose.

The scalp was often the first area to develop an itchy scaly erythema, which then spread over the pinnae, face, neck and upper limbs. In six cases a bright red, desquamating erythema covered most of the trunk and limbs. Response to local treatments, including corticosteroids, was slight. In twelve cases it was possible to stop the drug and when necessary substitute other anti-hypertensive agents. Eleven recovered completely within 2–3 weeks. The other dramatically improved, but had a history of eczema in childhood and some lesions remained. In seven patients it was considered justifiable to administer test doses of the drug, and six of these had a recurrence of their eruption, itching usually returning after one dose. Administration of the full daily dose produced unequivocal relapse.

REFERENCE

CRISSEY, J.T., OSBORNE, E.D. & JORDAN, J.W. (1957) Eczematous complications in treatment of tuberculosis. Eruptions resulting from combined aminosalicylic acid, dihydrostreptomycin and isoniazid therapy. *Journal of the American Medical Association*, **165**, 1526.

A painful bleeding syndrome associated with increased fibrinolytic activity

N.R.Rowell

Skin Department, The General Infirmary, Leeds

Two female patients with painful haemorrhagic episodes have been extensively investigated. The only haematological abnormality was an increase in fibrinolytic activity. Each patient was helped by treatment with aminocaproic acid.

The first patient, aged 56, gave a 23-year history of episodes of very painful haemorrhagic bursitis in various bursae, sometimes preceded by painful bruising of the skin, and occasional episodes of swelling and tenderness of joints. Tests for autoerythrocyte sensitivity and sensitivity to DNA were negative. Her euglobulin lysis time was consistently decreased and her plasminogen activity level slightly increased. Systemic prednisone made the condition worse, but aminocaproic acid for a period of 6 weeks gave permanent relief. She suffered a deep venous thrombosis in the course of therapy, but this resolved spontaneously.

The second patient, a woman of 57, developed episodes of painful bruising in 1971, and was also found to have a decreased euglobulin lysis time. Treatment with aminocaproic acid every 2 weeks has controlled the bruising, but there is no consistent relationship to the euglobulin lysis time.

Only one possible similar case has been found in the literature. It is suggested that these patients have a fluctuating level of plasminogen activator, and that if a bursa, a joint or skin is mildly traumatized bleeding will occur.

Transitional forms between dermatitis herpetiformis and bullous pemphigoid

S.Jablonska and T.Chorzelski

Department of Dermatology, Warsaw Medical School, Poland

Immunofluorescent (IF) studies have been performed in bullous diseases which, according to clinical, histological (skin and jejunal) and therapeutic criteria, corresponded either to bullous pemphigoid (BP) or dermatitis herpetiformis (DH). Circulating anti-basement membrane antibodies and *in vivo* bound immunoglobulins in the skin (IgG, IgA and IgM) have been determined in thirty cases of BP, twenty-four cases of adults DH, and in eleven cases of juvenile. Different immunological phenomena have been found repeatedly in BP and DH: anti-basement membrane antibodies and IgG bound *in vivo* to the epidermal-dermal zone in BP, and granular, fibrillar, or linear IgA deposits, and no detectable anti-basement membrane antibodies in DH.

The aim of the report is to present the transitional cases in which immunological phenomena were rather of BP type (linear IgG and IgA deposits; with prevalence of the former or the latter), clinical and histological pictures were variable (large bullae or a vesiculo-papular eruption, with or without micro-abscesses histologically), and the response to sulphapyridine or sulphones was dramatic, as in typical DH.

Transitional forms (or the mixed type) are more frequent in children: in our material five of eleven cases were of transitional type.

Although in the vast majority of BP and DH cases there are characteristic immunological features, an overlap of the clinical and immunological findings typical of the two diseases may occur.

Pemphigus and pemphigoid serum patterns in malignant tumours, benign tumours and normal skin

T.M.Phillips, J.Everall and D.Viza

Skin Department, Royal Marsden Hospital, London

This presentation is a study of the fate of naturally occurring skin antigens in malignant, pre-malignant and benign skin tumours.

It has been shown that normal skin contains specific components which are recognized as antigens only in the conditions of bullous pemphigoid and pemphigus vulgaris. In the former, the antibody is directed against the basement membrane, whilst in the latter there are antibodies against the intercellular substance (ICS) of epidermal squames.

In this study, we used both pemphigoid and pemphigus sera as markers to locate the presence of the antigens.

Biopsies both of lesions and of normal skin were screened by the indirect immunofluorescent technique, using both pemphigoid and pemphigus antibody, at a standard titre, to detect the degree of antigenicity within the specimen.

The results showed that both of the antigens were present in normal skin, atrophied skin, 10–16 week embryonic skin and benign tumours, but were reduced to a variable degree in pre-malignant and malignant lesions, depending on their type, duration and degree of infiltration.

Explanations for this antigenic variability, in malignancy, are, (1) the antigen is definitely lost or completely displaced as the cells become involved, (2) the antigen is altered sufficiently so as to be unrecognizable to the antibody. This process is gradually performed as the disease progresses.

Complement in the skin of patients with dermatitis herpetiformis

P.P.Seah, M.R.Mazaheri and Lionel Fry

Department of Dermatology, St Mary's Hospital, London

Forty-two biopsies from twenty patients with dermatitis herpetiformis (DH) have been examined by immunofluorescence, for IgA, IgG, IgM, and C1q and C3 components of complement. Both the involved and uninvolved skin have been studied.

All forty-two biopsies showed IgA in the dermal papillae or blood vessels in the dermis. In addition, three specimens had IgM and one IgG deposits. Twenty-four of the forty-two biopsies showed the presence of C3 in the dermal papillae or dermis. In the majority of these cases the distribution of the C3 deposits, by immunofluorescence, corresponded to that of the IgA deposits. C1q deposits were found in only three of the biopsies, which also had IgG or IgM in them in addition to IgA.

The results show that complement is activated in the skin in dermatitis herpetiformis and is found irrespective of whether clinically involved or uninvolved skin is examined, or whether the patient is on therapy. The immunofluorescent studies indicate that IgA is the main immunoglobulin involved with C3, and the absence of C1q in the skin (except in three cases) would suggest that complement activation in DH is mainly via the 'alternate pathway' as opposed to the 'classical pathway'. This concurs with the current concept that IgA, probably in an aggregated form, activates complement via the 'alternate pathway'. It would appear that the lesions in DH are the result of immune complexes. The role of dapsone in the control of DH is uncertain. These results show that it does not block complement activation at the C3 level, but further work needs to be done on the later components of the complement cascade before its role as a possible immunological blocker can be fully assessed.

Functional and morphological changes in response to a gluten-free diet and corticosteroids in dermatitis herpetiformis and adult coeliac disease

PARVEEN J.KUMAR, D.B.A.SILK, R.MARKS, M.L.CLARK AND A.M.DAWSON

Departments of Gastroenterology and Medicine, St Bartholomew's Hospital, and St John's Hospital for Diseases of the Skin, London

Patients with dermatitis herpetiformis (DH) are now being treated with a gluten-free diet, and we have therefore undertaken a study to correlate the jejunal function with the mucosal abnormality in these patients and a comparable group of patients with adult coeliac disease (ACD). As corticosteroids have also been shown to improve the jejunal morphology of patients with ACD we have studied the response to this treatment in patients with DH.

Twenty-three patients with DH and nine with ACD were studied. Jejunal morphology was assessed histologically on biopsies obtained with a Crosby capsule. Function studies, performed using the double lumen perfusion technique, involved the study of glucose, water and electrolyte absorption from a 56 mM glucose solution.

In the untreated groups, varying degrees of functional impairment were seen which could be related to mucosal structure. In normal subjects, absorption of water and electrolytes occurs in the presence of intraluminal glucose, but in the ACH group and some of the DH patients actual secretion of fluid occurred.

Treatment with a gulten-free diet and corticosteroids in both diseases produced a marked improvement in morphology as well as function; in many cases there was a reversal of the secretory state.

These findings emphasize the similarities between the enteropathies of ACD and DH, and further substantiate the value of a gluten-free diet in these patients.

Immunofluorescence of the jejunal mucosa in dermatitis herpetiformis and adult coeliac disease

M.LANCASTER-SMITH, PARVEEN KUMAR, R.MARKS, M.L.CLARK AND A.M.DAWSON

Departments of Gastroenterology and Medicine, St Bartholomew's Hospital, and St John's Hospital for Diseases of the Skin, London

In view of the similar jejunal morphological lesions found in adult coeliac disease (ACD) and dermatitis herpetiformis (DH), a study has been undertaken to compare the immunoglobulin containing cells of the jejunal mucosa in both conditions. Direct immunofluorescent techniques were used for studies on the jejunal mucosa of eighteen patients with DH who had varying morphological changes, thirteen patients with ACD, and ten control subjects with normal morphology. Some of these patients were on a gluten-free diet. Serum and jejunal juice immunoglobulin concentrations were also measured using an immunodiffusion method.

Comparison with control subjects revealed a marked increase in IgM-containing cells in both DH and ACD. In all three groups the IgA- and IgM-containing cells were distributed throughout the lamina propria, the highest density of the former occurring at the bases of the villi, whereas the latter cells were most numerous deeper in the lamina propria. There was no correlation between plasma cell abnormalities with either jejunal morphology or treatment with a gluten-free diet. In patients with ACD and DH, one-third had low serum IgM levels, but this did not correlate with the quantity of IgM-containing cells in the intestinal mucosa.

The finding of increased numbers of IgM-containing cells in ACD is in agreement with other workers. This is the first study to quantitate immunoglobulin-containing cells of the jejunal mucosa in dermatitis herpetiformis. The demonstration of a comparable abnormality to that of ACD lends support to the concept that the small bowel lesion in both disorders is of similar aetiology.

IgE and diseases of the skin

R.K.Winkelmann, G.J.Gleich, S.A.Muller and R.E.Jordan

Mayo Clinic, Rochester, Minnesota, U.S.A.

Immunoglobulin E values have been most consistently elevated in atopic dermatitis. The correlation of extent and severity of disease with the titre of IgE has been observed, but relationship to other features of atopy is lacking. The description of a chronic, acral keratotic dermatitis with massive IgE blood values and without atopy indicates that other forms of skin disease may show an increased response of this immunoglobulin system. Preliminary results indicate that bullous pemphigoid may be such a disease. The results of our studies of atopic, non-atopic dermatitis and bullous pemphigoid will be discussed in relation to IgE determinations from over 500 patients with diseases of the skin.

Skins in the East

John H.S.Pettit

Room 303, China Insurance Building, 174 Jalan Tuanku Abdul Rahman, Kuala Lumpur, Malaysia

A number of cases will be discussed, all of which have been referred for consultant opinion because of the doctor's suspicion of leprosy. Some of the conditions are well-known in the United Kingdom, but others are more frequently seen in Asia.

Changes in cutaneous blood flow after ultraviolet radiation at 250 nm and 300 nm

C.A.Ramsay and A.V.J.Challoner

Institute of Dermatology, Homerton Grove, London

This investigation was carried out using photoelectric plethysmography (PEP), skin thermometry and a thermal conductance method. Cutaneous blood flow was measured in eight subjects at the two wavelengths at regular intervals after irradiation. Measurements were made at five times the minimal erythema dose (MED) with PEP, and at two and five times the MED with both thermal conductance and skin thermometry.

Results. At both wavelengths erythema was occasionally seen at 3 h and always at 6 h. Skin thermometry and PEP indicated an increase in blood flow at both wavelengths at 3 h and at each subsequent reading for several days. The thermal conductance method showed differences between doses of two and five times the MED. At twice the MED with both wavelengths, an increase in flow was present at all readings until 72 h, whereas at five times the MED, an initial increase in flow preceded a decrease which became evident at 24 h. A possible explanation of the differences in blood flow observed at five times the MED by the different methods is that PEP measures blood flow in the whole of the dermis whilst thermal conductance measures changes only in the superficial dermis.

Conclusions. (1) Blood flow in the skin is increased after 250 and 300 nm irradiation; a finding in direct contrast to other reports. (2) Larger doses of ultraviolet radiation lead to a reduction in flow in the upper dermis, probably due to stagnation of blood in the superficial capillaries. (3) There is probably only one mechanism responsible for the erythema induced by different wavelengths of ultraviolet radiation.

Chronic dermatitis with photosensitivity and its relationship to actinic reticuloid

T.LAKSMIPATHI, JOYCE HERD, J.ROGERS AND W.FRAIN-BELL

Department of Dermatology, University of Dundee

We wish to report the results of a study of chronic dermatitis in twenty-seven male subjects in whom there was clinical photosensitivity, and in some instances morphological evidence of actinic reticuloid (Ive *et al.*, 1969). The clinical, histological and photobiological findings will be reported along with a discussion of the relationship between chronic dermatitis plus photosensitivity and actinic reticuloid.

Method

The photobiological studies were carried out using a 1600 W xenon arc light-source with a diffraction grating monochromator (Mackenzie & Frain-Bell, 1973) and biopsies were taken from clinically affected skin as well as from irradiation sites on the skin of the back.

Results

Four cases were discarded because clinical photosensitivity was doubtful and normal action spectra for erythema were obtained. The remaining twenty-three patients were all males, having suffered from dermatitis, usually of exposed sites, for longer than 5 years in fourteen instances (60%).

Photobiological. The abnormal and action spectra for either erythema or papular response were found to be in the short sunburn UVR wavelengths (<320 nm) in all cases (100%), in the short and long UVR wavelengths (290–400 nm in twenty-one cases (90%), and to involve visible light wavelengths as follows: at 400 nm (370–430 nm) in fifteen cases (65%); at 500 nm (470–530 nm) in six cases (26%); and at 600 nm (570–630 nm) in three cases (13%).

The histological findings from biopsies taken from clinically affected skin in twenty subjects showed changes of actinic reticuloid in eleven instances, and those of chronic dermatitis in nine. Of ten cases, in whom the clinical and photobiological studies strongly suggested actinic reticuloid, the histological features of biopsies taken from irradiated sites supported this in eight instances, being non-specific in two. In four subjects with apparent chronic dermatitis and photosensitivity, the histology of the irradiated site was non-specific.

Treatment

The treatment of these cases consisted of topically and/or systemically administered corticosteroid preparations along with steps to reduce exposure to light from both natural and artificial sources (e.g. fluorescent lighting) using appropriate light screening agents, with nursing in a special polythene cubicle in the more acute cases (Herd, Sturrock & Frain-Bell, 1973).

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Chloropromazine phototoxicity: a non-classical mode of action

B.E.JOHNSON

Department of Dermatology, University of Dundee

Kahn & Fleischaker (1971) showed chloropromazine to be a phototoxic compound in studies using red blood cell (RBC) haemolysis as an experimental model. This paper reports more detailed studies of chlorpromazine in this model system, and the application of the results to explanation of skin reactions to chlorpromazine.

A washed RBC suspension, diluted to 1 in 500 in 2 mg/100 ml chlorpromazine (both RBC suspension and drug solution made up in barbitone buffered saline, 0·9% pH 7·2) showed 15–20% drug uptake and minimal dark haemolysis. 10 ml of this mixture in 82 mm diameter petri dishes, providing a monolayer of RBCs, was irradiated with a battery of Westinghouse FS20 sunlamp fluorescent tubes through a plastic + water filter giving 10% cut-off at 315 nm. 20–40 min exposure gave 100% haemolysis, while dark controls and RBCs alone gave only minimal haemolysis. Classically, drug induced photohaemolysis involves only that drug taken up by the cells. When excess chloropromazine was removed, a requisite for accurate action spectrum studies, and replaced by buffer, no photohaemolysis could be obtained. Instead, a 2 mg/100 ml solution of chlorpromazine irradiated for 40 min prior to the addition of RBCs produced 100% haemolysis. In this model system, a toxic photoproduct is formed, which alone acts on the RBC membrane. No binding of parent compound or energy transfer from it is required.

Using a grating monochromator with half maximum bandwidth of 10 nm, an action spectrum above 290 nm for the effect, irradiating only the chlorpromazine, shows good correlation with the absorption spectrum of chlorpromazine, with a peak at 305 nm and decreasing activity towards longer wavelengths of the ultraviolet spectrum.

The mechanism in reactions to intradermal injections of chlorpromazine followed by irradiation (Hunter, Bhutani & Magnus, 1970) may be similar to that for photohaemolysis. However, a 1 in 200 dilution of serum, added to the drug prior to irradiation or to the RBCs prior to addition of irradiated drug, inhibits haemolysis. Nonetheless, in nine of twelve mice, skin reactions were produced by intradermal injections of 0·2 ml of irradiated chlorpromazine 50 mg/100 ml, and not by non-irradiated chlorpromazine.

These results indicate that phototoxicity studies should include irradiation of the chemical before incorporation into the test system if the mechanism of action is to be understood.

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KAHN, G. & FLEISCHAKER, B. (1971) Red blood cell haemolysis by photosensitizing compounds. *Journal of Investigative Dermatology*, 56, 85.

Woolly hair

P.E.HUTCHINSON AND R.S.WELLS

St John's Hospital for Diseases of the Skin, London

Six families, in which one or more individuals had 'woolly hair', were investigated. In addition to clinical and genetic studies, the abnormal hair was examined with the light and scanning electron microscopes. A trichogram was constructed for each patient, that is the hair diameter and hair density per unit area were measured, and growth rate and the anagen–telogen ratio were estimated. Initially they were classified into three groups depending on their mode of inheritance and the clinical features.

First, there were two families where the mode of inheritance was that of an autosomal dominant trait. For these the name Hereditary Woolly Hair is suggested. Affected individuals had tight curls in childhood and very wavy hair as adults. No other clinical abnormality was noted. Microscopical examination showed torsion of the hair about the long axis, and an ovoid cross section. The hair diameter was decreased; the hair density, the growth rate and anagen–telogen ratio were all normal, but there was a large proportion of dysplastic hairs.

Secondly, genetic evidence pointed to an autosomal recessive mode of inheritance for three of the patients. For these and other individuals recorded in the literature with the same mode of inheritance, the name Familial Woolly Hair is suggested. The findings in this group were similar to those in the first, except that they were all very fair, they were more severely affected and the body vellus hair was abnormally short. The periodicity of the longitudinal twisting was shorter and the hair diameter was smaller than in the first group.

Thirdly, one patient was investigated who had a Woolly Hair Naevus. This is probably an acquired localized developmental abnormality. Hair taken from the abnormal area closely resembled that of patients in the second group.

Preliminary observations of an immunologically determined dermatosis in reptilian skin homografts

I.W.WHIMSTER

Department of Dermatology, St Thomas' Hospital, London

When homografts of full thickness skin are exchanged between normal adult reptiles these grafts invariably are completely rejected, within weeks or at most a few months.

In an attempt to obtain successful exchanges of skin homografts, the procedure was carried out in pairs of juvenile sibling geckos within 24 h of hatching. It was found that homografts exchanged at this age do one of three things.

(1) Some are totally rejected, as in adults.

(2) Some are totally accepted and behave as if they were autografts. They grow with the host animal and have remained without visible abnormality for periods of up to 3 years.

(3) The third group of homografts are 'partially accepted', in that the skin forming the graft remains morphologically intact and grows with the host animal, but it undergoes certain progressive changes. These most conspicuously affect its pigment cells, which are either lost or cease to function, so that the graft becomes and remains colourless, as though the host had a persistent 'hostility' towards pigment cells.

Since this phenomenon has never been observed in skin which has been autografted, it is concluded that the loss of pigmentation is not simply the result of ischaemia or any mechanical failure of the actual grafting process, but that it is the outcome of an immune reaction causing 'incomplete rejection'.

If this interpretation is correct, it is an unusual and interesting reaction, in that it appears to affect predominantly only certain cells of the graft; conspicuously its pigment cells. The epithelial and connective tissues of such grafts have remained intact after a period of more than 2 years.

This experimental situation, which should be capable of expansion and analysis, has certain obvious parallels to vitiligo and various other human dermatoses.

Freeze-fracture replication of melanocytes and melanosomes

A.S.BREATHNACH

Department of Anatomy, St Mary's Hospital Medical School, London

The technique of freeze-fracture replication permits examination at the ultrastructural level of essentially viable tissue which has not undergone conventional processing. It provides a new dimension for studying the functional morphology of cells and organelles, and particularly of membranes and membrane limited structures.

This communication presents preliminary results of the application of the technique to study of the melanocyte and melanosome. Epidermal melanocytes of mature and foetal human skin, and retinal and choroidal melanocytes of the guinea-pig and human foetus were examined. Melanosomes are identified in replicas mainly by their shape and size. The majority fracture so as to reveal complementary fracture faces of the limiting membrane, and features of these faces can be distinguished from those of fracture faces of other cytoplasmic organelles. Fully mature melanosomes seen in cross-fracture exhibit no organized internal structure. The same, surprisingly enough, applies almost invariably to immature melanosomes of foetal material. This indicates that the concentrically arranged lamellar substructure seen in stained thin sections is probably not membranous in nature.

Secondary syphilis: a clinico-pathological reappraisal

E.ABELL, R.MARKS AND E.WILSON JONES

St John's Hospital for Diseases of the Skin, and The Institute of Dermatology, London

Over the last two decades the average number of patients presenting annually at St John's Hospital with secondary syphilis has doubled. During this period, of the confirmed biopsied cases, the diagnosis was clinically unsuspected in 10% and was not considered to be the most likely one in a further 15%. Clinical confusion has arisen most frequently with pityriasis lichenoides, but also with toxic erythema, psoriasis and eczema.

In order to define the distinguishing histological features, biopsies of sixty cases of secondary syphilis seen since 1952 have been re-examined and correlated with the clinical state and stage of disease.

The histological patterns of the secondary eruption are determined not only by the morphology of the biopsied lesion, but also by the duration of cutaneous involvement. Although no single change is constantly present to distinguish this condition, the following features require emphasis:

(1) Epidermal invasion by inflammatory cells usually occurs at an early stage of the disease.

(2) The epidermis usually shows hyperplasia and psoriasiform change in the later stages of the condition.

(3) The 'wedge' shaped pattern of the dermal inflammatory reaction suggests involvement of medium sized vessels.

(4) A granulomatous inflammation is seen with predominance of lymphocytes and histiocytes in varying proportions.

Prominent endarteritis and marked plasma cell infiltration are less frequently present than most accounts of secondary syphilis would indicate.

The epidermis in pityriasis rubra pilaris: a comparison with psoriasis

R.MARKS AND A.GRIFFITHS

St John's Hospital for Disease of the Skin, and The Institute of Dermatology, London

Pityriasis rubra pilaris (PRP) is an uncommon chronic scaly dermatosis of unknown cause with marked clinical similarities to psoriasis. As PRP has a distinctive clinical course and may require treatment differing from that used in psoriasis, it would be useful to be able to distinguish it in the laboratory. With this end in view, we have studied twenty biopsies from patients with unequivocal classical PRP. To obtain information concerning the cell kinetics of PRP, we have studied lesions from nine patients autoradiographically using tritiated thymidine, and in addition examined six PRP lesions by enzyme histochemical methods. We have contrasted our histological, enzyme histochemical and cell kinetic findings with the results of similar investigations in a matched group of psoriatic patients, and have made the following conclusions:

(1) The rate of cell proliferation of PRP lesions is raised, but does not reach the level seen in psoriasis. This is also reflected in the reactivity in the histochemical tests for mitochondrial enzymes.

(2) In contrast to psoriasis, there appear to be increased numbers of dendritic cells within the epidermis in the lesions of PRP, *pari passu* with the keratinocyte hyperplasia.

(3) Apart from the overall architectural differences between the lesions in the two diseases, there appear to be specific differences in the parakeratotic horn, and an unusual perinuclear vacuolation in the Malpighian layer in PRP.

It is suggested that, on the basis of these findings, PRP can be distinguished from psoriasis, despite close clinical similarities.

The regenerative capacity of the basement membrane region

E.ABELL, T.NISKIKAWA AND R.MARKS

St John's Hospital for Diseases of the Skin, and The Institute of Dermatology, London

The dermo-epidermal junction is of cardinal importance in many skin disorders, including the blistering diseases, yet little is known about either the development or regeneration of this area.

The aim of the present study has been to investigate the way in which the junctional region regenerates in a wound healing 'model'. This 'model' utilizes the migration of epidermal cells from the margins of skin fragments maintained in an artificial medium, and has been described in detail elsewhere (Marks, Bhogal & Dawber, 1972).

Altogether 150 explant fragments of human skin were cultured for periods of 1–5 days, and then examined for:

(1) The development of antigenic components of basement membrane by immunofluorescent techniques, after incubation of sections with pemphigoid serum.

(2) The presence of connective tissue components of the junctional zone, using the PAS reaction and silver stains to detect reticulin.

(3) The development of a basal lamina beneath migrating epidermal cells, using ultrastructural techniques.

Epidermal cell migration occurred in 90% of the specimens. All components of the normal junctional zone were present beneath the epidermis of the central body of the explant, but absent beneath the migrating epidermis in the first 48 h of culture. By 72 h the basal lamina was present beneath the migrating epidermis in some specimens, and a positive fluorescent band was also sometimes apparent at this time. In skin maintained for longer periods, it became evident that the basal lamina and the positive fluorescent band were necessary before maturation of the migrating epidermis could take place.

In conclusion, it appears that the basal lamina and antigenic components of the junctional zone develop beneath regenerating epidermis sometime after epidermal repopulation of the injured area. Furthermore, these results suggest that epidermal cells themselves produce these two elements of this zone, but that the PAS basement membrane derives from the dermis.

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CASE REPORTS

(1) Primary macroglobulinaemia and lymphoma

DR A. NAVARATNAM AND DR G.A. HODGSON

Department of Dermatology, University Hospital of Wales, Cardiff

O.A., female, aged 53 years.

History. Her first symptoms were intermittent spells of fatigue and unusual bodily feelings, such as being '8 ft tall', or 'having taken three dry sherries on an empty stomach'. This began in 1966. She was

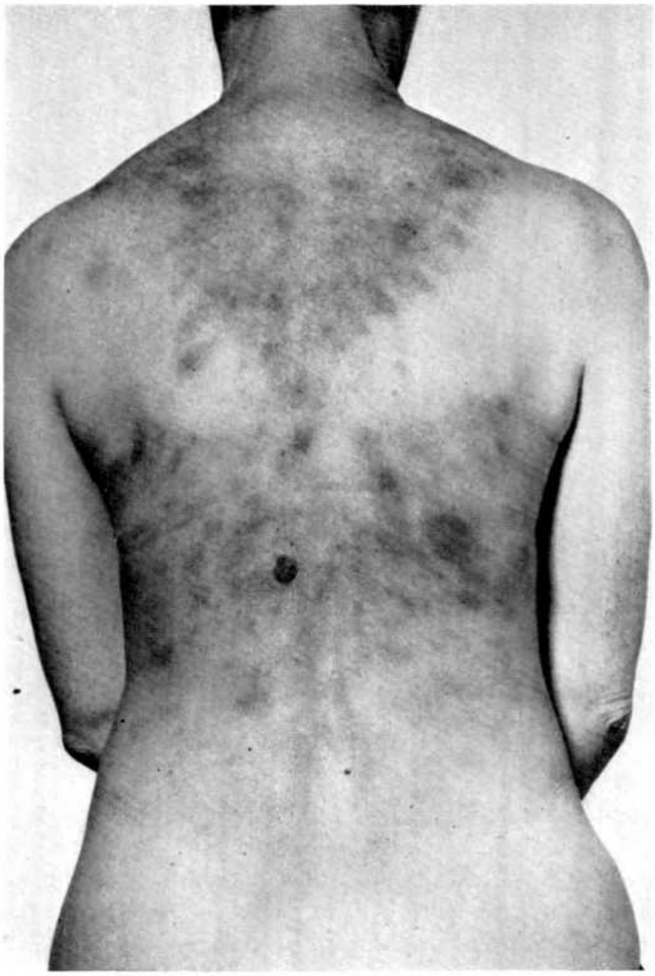

FIGURE 1. Diffuse areas of infiltration by lymphoma on back.

then found to have a raised ESR (145 mm/1h); and primary macroglobulinaemia was diagnosed on electrophoresis.

In 1968, with the return of odd feelings ('cerebral wooliness') and loss of weight, faint macular discoloured patches appeared on the chest, abdomen and forehead. Later flat plaques were formed which have recently become darker and slightly elevated in some areas.

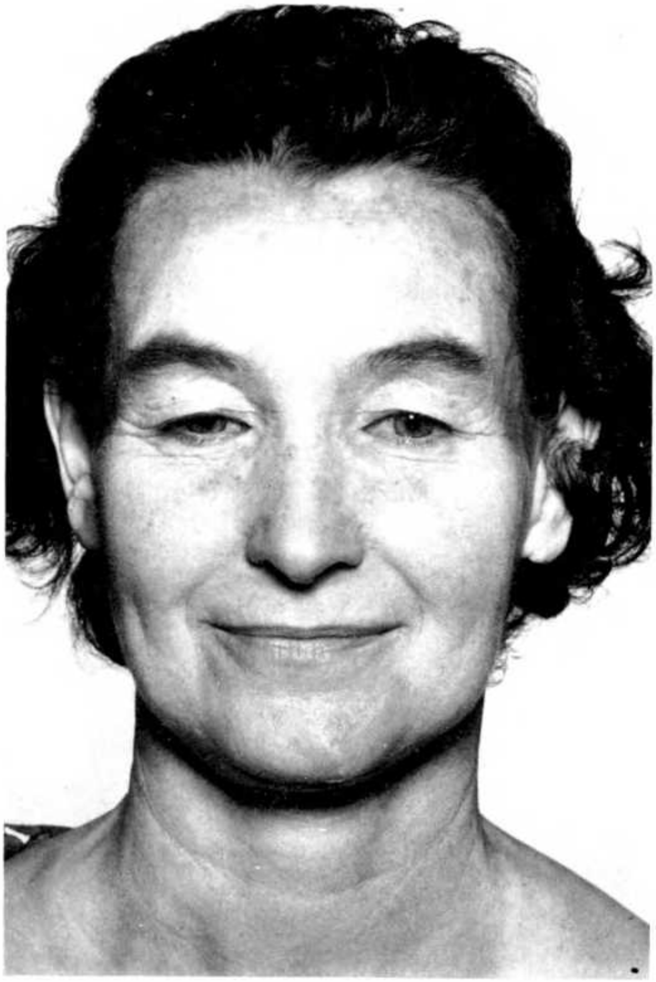

FIGURE 2. Band type discolouration from infiltration along forehead.

On examination. Patchy, macular pigmentation and dark brown flat or raised plaques on the chest, back, abdomen (Fig. 1), with a band of discoloured infiltration on the forehead (Fig. 2) and plaques on the sides of the face and cheeks. There is bilateral axillary lymphadenopathy and palpable enlargement of the liver and spleen. Other systems normal.

Investigations. Urine. No Bence-Jones proteins or proteinuria.

Blood. Hb. 9·7 g/100 ml. WBC 4000/mm^3 (neutrophils 2400/mm^3, lymphocytes 17,060/mm^3). Serum electrophoresis (1966); abnormal serum protein pattern with marked band in globulin fraction

(Fig. 3). Platelets 327,000/mm^3. Coombs test negative. ESR 154 mm/1 h (1966), 147 mm/1 h (1970). EEG (1966) normal. Bone marrow; iliac crest and sternum (1970)—'a very hypocellular specimen. Marrow cells were present and the lymphocytes numbered about 20% of the total. A number of the lymphocytes were atypical. Findings consistent with a diagnosis of macroglobinaemia'. Electrolytes, bilirubin, alkaline phosphatase, urea and cholesterol normal. Total proteins 8·6 g%. Albumin 3·5 g%. Gammaglobulins 3·6 g% (normal 0·6–1·5 g%). Immunoglobulins: IgA 5 mg/100 ml (normal 250± 100 mg/100 ml); IgG 500 mg/100 ml (Normal 1300±500 mg/100 ml); IgM 500 mg/100 ml (Normal 130±50 mg/100 ml). Antinuclear antibodies not detected.

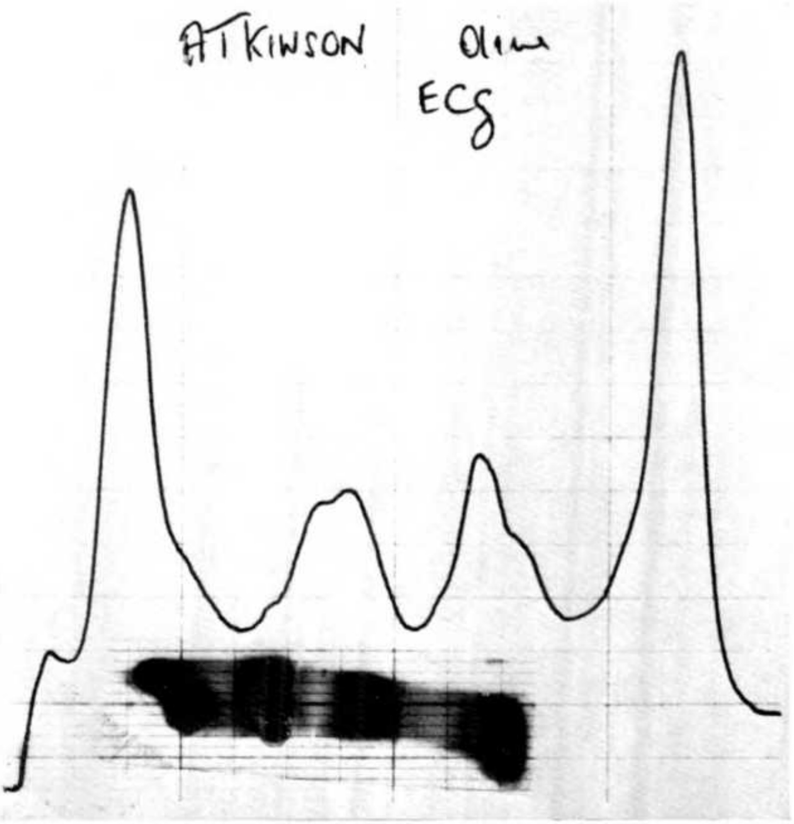

FIGURE 3. Protein electrophoresis, with marked band in globulin fraction.

X-ray. Skeletal survey NAD

Biopsies. (a) *Lymph node* (1970). Architecture of the node is not destroyed, but follicles extend throughout its substance. The perinodal fat is densely infiltrated with lymphocytoid cells resembling those in the follicles and with larger pyrinophilic cells. The latter are also seen in abundance within the follicles and the nodes. Appearances are those of macroglobulinaemia. (b) *Skin biopsy* (1972) (Fig. 4). Dermis extensively infiltrated with a poorly differentiated tumour of the lymphoma group. Since cells are primitive it is not really possible to say to which cell type they belong. (c) *Jejunal biopsy* (performed because of low serum folate), normal.

Course and treatment. The skin lesions are getting more marked and larger, fusing into plaques, especially over her back and forehead, with recession of the hair line. Topical steroids have not altered the skin infiltration. No other specific therapy except treating her anaemia with iron supplements.

Discussion. Waldenstrom (1944, 1948) described primary macroglobulinaemia as 'incipient myelomatosis or essential hyperglobulinaemia with fibrinogenopenia', later calling it macroglobulinaemia (Waldenstrom, 1948). It is a plasma cell dyscrasia with an excessive proliferation causing the elaboration of the large quantities of gammaglobulin found on electrophoresis.

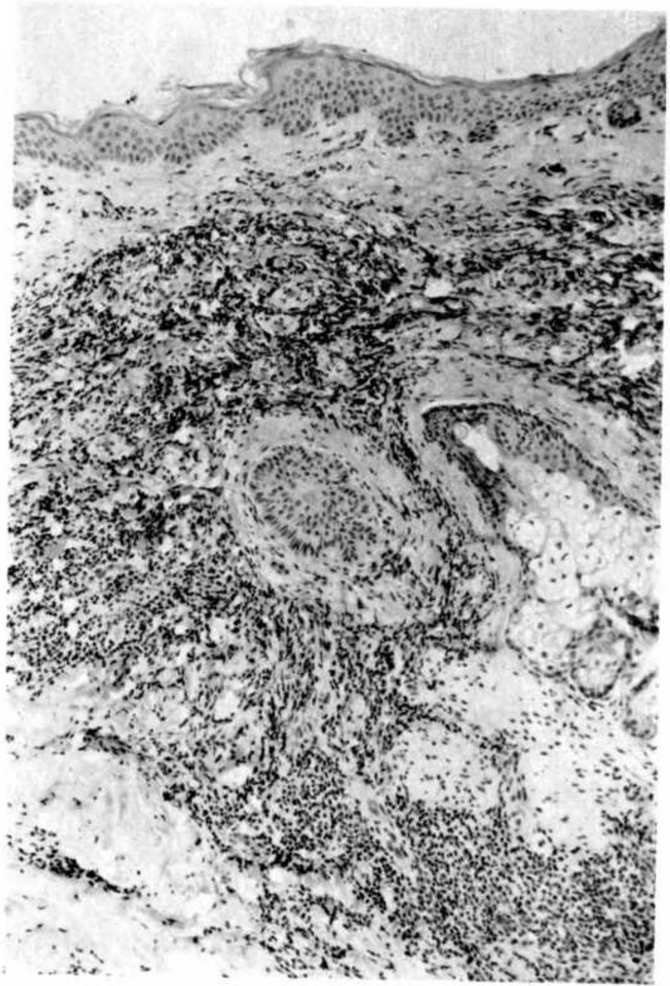

FIGURE 4. Biopsy of skin. Diffuse infiltration of dermis with poorly differentiated tumour of lymphoma group composed of primitive cells (×94).

The main clinical features are anaemia, bleeding, hepatosplenomegaly, and the raised ESR. The skin changes hitherto described in primary macroglobulinaemia were mainly due to the bleeding diathesis; classically as recurrent purpura of the legs leaving pigmented discolouration. Other reported findings are haemorrhagic phenomena in two thirds of the cases reported by Schulten & Kanzow

(1956), and brown pigmentation of the skin, with histological evidence of para-amyloid deposits containing Bence-Jones proteins (Mandema, Vander Schapf & Huisman, 1955).

This patient with extensive discoloured skin involvement of the trunk, face and forehead, is a different picture from 'purpura hyperglobulinaemia Waldenstrom' (Waldenstrom, 1948).

The skin shows areas of poikiloderma and infiltration, clinically meriting the term 'incipient', but as a reticulosis instead of a myelomatosis.

Histology showed the dermis to be infiltrated extensively with poorly differentiated tumour cells of the lymphoma group. Since the cells were primitive, it is not possible to say to which cell type they belong.

The primary type of macroglobulinaemia almost certainly reflects the presence of a 'neoplastic disease', in some respects resembling lymphoma, and in other respects a myeloma (Ritzmann *et al.*, 1960).

In approximately 8% of all cases with macroglobulinaemia of Waldenstrom carcinomatous lesions were encountered (Kappeler, Krebs & Riva, 1958).

In most cases, however, the time relationship as to the occurrence of a carcinoma and macroglobulinaemia has not been established. One may therefore assume that some of these cases represent secondary macroglobulinaemia (Ritzmann *et al.*, 1960).

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(2) Lymphocytic lymphoma in a patient with diabetes mellitus

DR R. W. DAVIES AND DR G.A.HODGSON

Department of Dermatology, University Hospital of Wales, Cardiff

P.H., male, aged 72 years. Retired regimental sergeant major.

History. The patient complained of a persistent irritable eruption on both soles for 3 years (Fig. 2). Elevated non-tender nodules were noticed on the forearms in Autumn, 1972, after exposure to sun. Griseofulvin was given by the general practitioner for a period of 9 months for presumed ringworm infection. The patient also received methyldopa about 1 year ago for hypertension.

On examination. There is early finger clubbing. There are three purple, elevated, firm, non-tender nodules on the flexor aspects of the forearms, about 4 cm in diameter. One lesion on the right forearm borders a tattoo (Fig. 1). There are multiple small purplish and hyperkeratotic maculo-papules on the

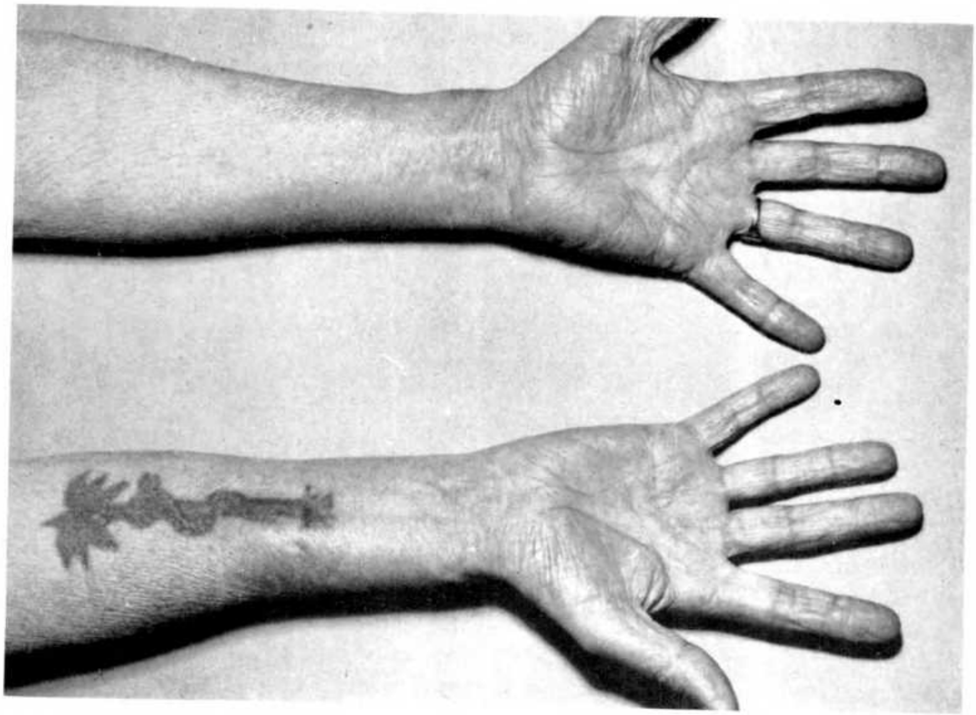

FIGURE 1. Note lesion bordering tattoo.

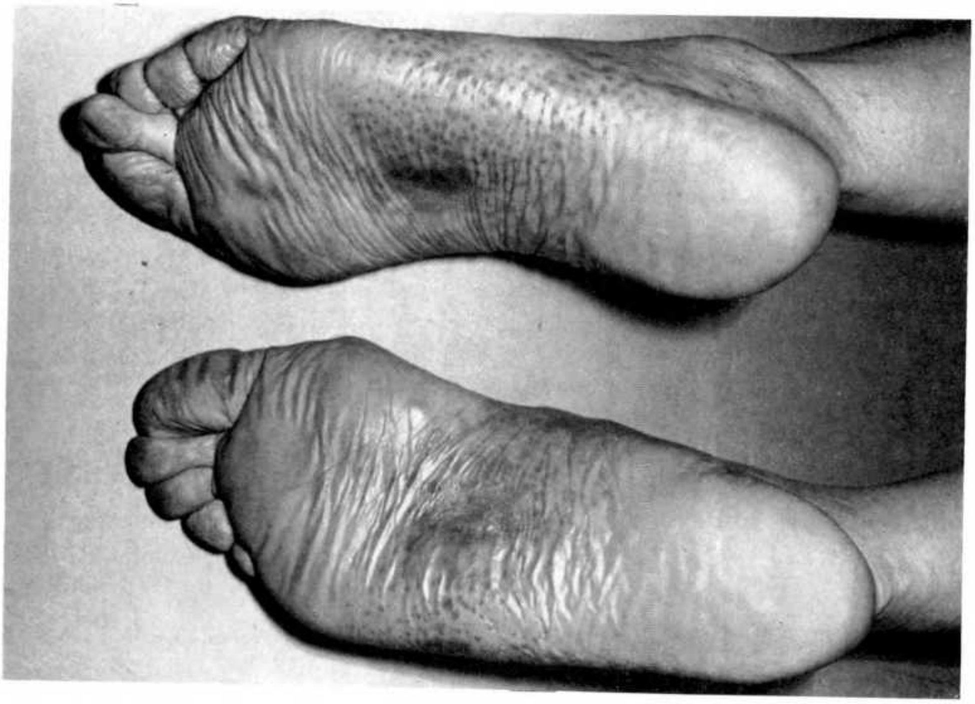

FIGURE 2. Maculo-papules on both soles.

soles (Fig. 2). The spleen, liver and cutaneous lymph nodes are not palpably enlarged. Mild hypertension and glycosuria are present.

Investigations. Blood. Hb 14·0 g/100 ml. WBC 6100/mm^3. Normal differential count and film. ESR 25–35 mm/1 h.

Biochemistry. Serum calcium, urea and electrolytes, protein and electrophoresis, all normal. Alkaline phosphatase 50 IU/l; (normal 10–90 IU/l). Bilirubin 23 μmol/l; (normal 0–17 μmol/l). Remainder of liver function tests normal. GTT confirmed diabetes mellitus. ANF negative on two occasions. Immunoglobulins normal. WR, negative. Mantoux positive on two occasions (1 : 10,000).

Biopsy. Skin. (a) Left sole; sections show dermal perivascular foci of lymphocytes and histiocytes (Fig. 3). No vasculitis. (b) Right forearm; consistent with benign lymphocytic infiltrate (Fig. 4).

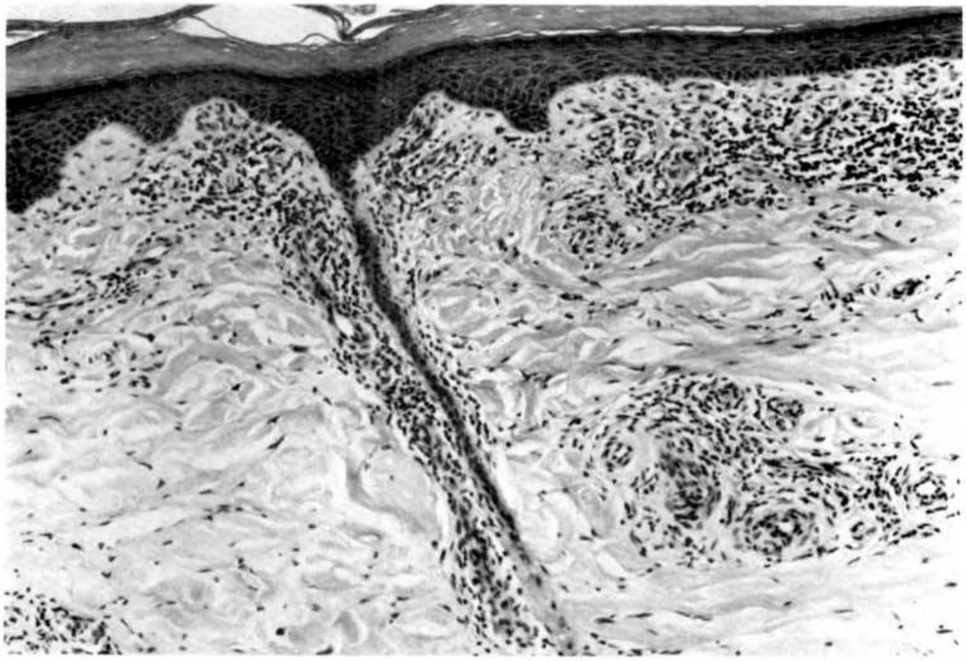

FIGURE 3. Hyperkeratinization of the epidermis with slight perivascular lymphocytic infiltration in the dermis (× 118).

X-rays. (a) Barium meal and follow through, normal. (b) Chest; widespread mottling (non-specific).

Course and treatment. Haelan tape on one forearm lesion for 6 weeks produced temporary regression during this time. Now, slowly progressing with irritation. Diabetes mellitus controlled on diet.

Comment. Histological examination shows a lymphocytic infiltrate which appears benign, as might be found in such conditions as benign lymphocytic infiltration (Jessner), or discoid lupus erythematosus. This histology excludes sarcoidosis. There is no follicular arrangement of the lesions and there is no epidermal involvement to support a diagnosis of discoid lupus erythematosus. Moreover, dermo-epidermal immunoglobulins were absent. Jessner's benign infiltration occurs mostly on the face, although other areas may be affected (Calnan, 1957), but it appears and disappears. A progressive malignant reticulosis may in its early stages be indistinguishable from a benign lymphocytic infiltrate (Calnan, 1957). In malignant lymphoma there is a relatively common association with such skin disorders as herpes zoster and exfoliative dermatitis (Lever, 1967); lymphocytic leukaemia may also occur in one third of cases (Soll & Mallory, 1942). None of these is a feature here. In spite of the benign nature of the infiltrate, the course of the disorder makes it likely that we are dealing with a malignant progressive lymphoma.

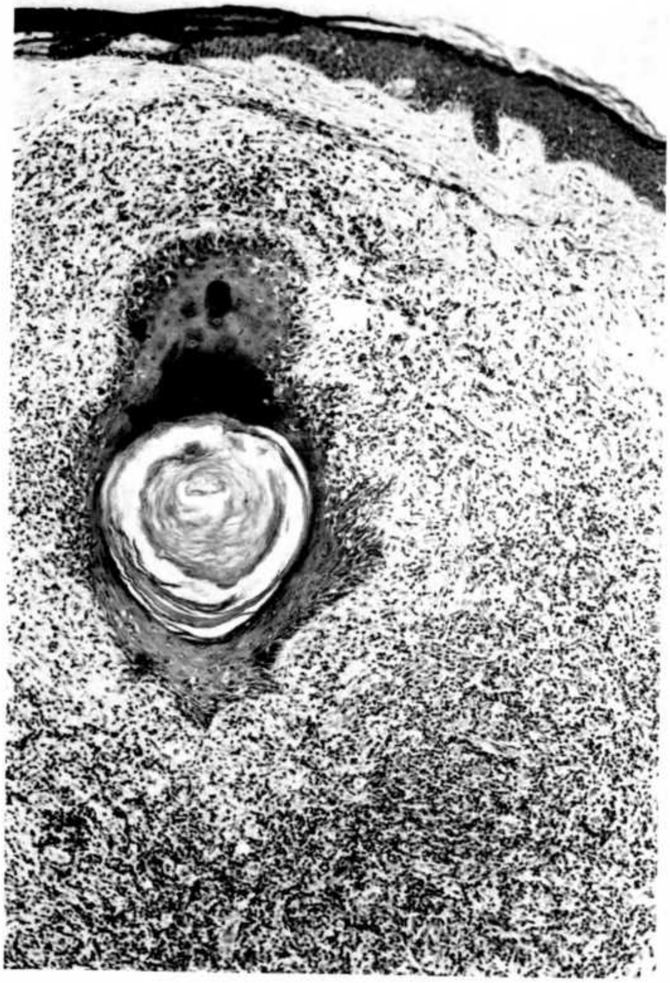

FIGURE 4. Dermal perivascular foci of mixed lymphocytes and histiocytes. No evidence of neoplasia. Consistent with 'benign lymphocytic infiltration' of the skin (× 38).

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(3) Recurrent cutaneous suppuration complicating acne with paroxysmal nocturnal haemoglobinuria (PNH)

DR P.J.A.HOLT AND DR G.A.HODGSON

Department of Dermatology, University Hospital of Wales, Cardiff

D.I.T., male, aged 25 years.

History. In August, 1967, aplastic anaemia was diagnosed after 3 months of dyspnoea, epistaxis and spontaneous bruising. He was treated with prednisolone 20 mg daily, and 1 month later, when aged 15, he developed acne vulgaris on the face, back and chest. Steroids were discontinued after 9 months. The pancytopenia persisted although the abnormal bleeding tendency improved.

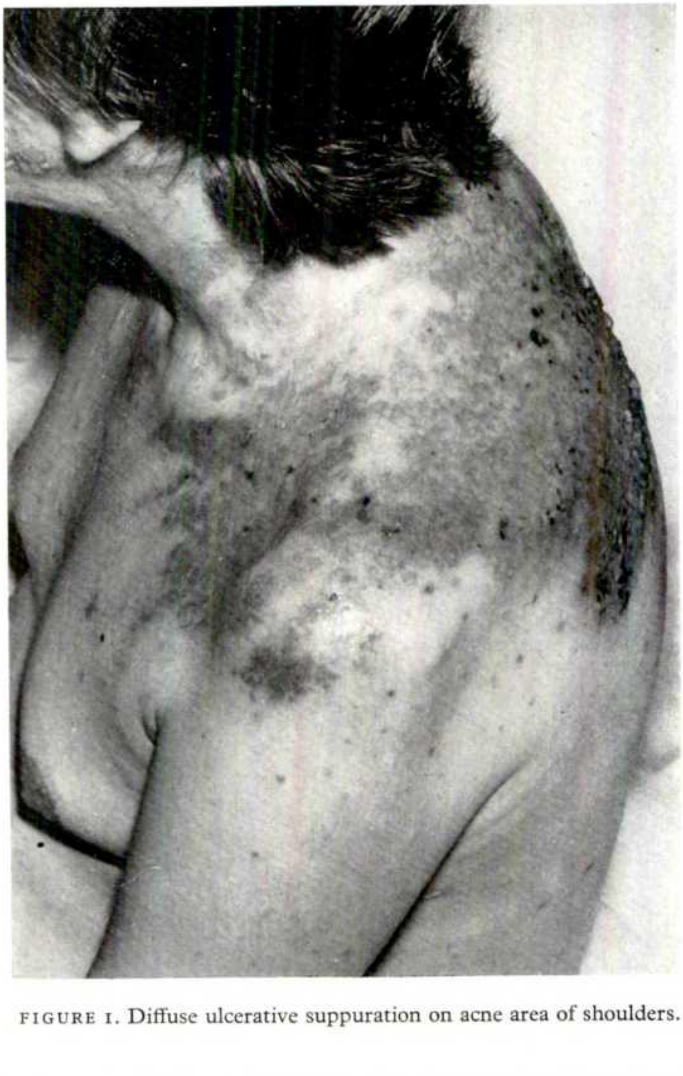

FIGURE 1. Diffuse ulcerative suppuration on acne area of shoulders.

March, 1970. Deep vein thrombosis of the right leg.

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June, 1970. An acute crisis with pancytopenia and reticulocytosis was diagnosed as PNH. He had more severe acne on the face and neck, with acne conglobata on the back and suppurative hidradenitis in the left axilla and the groins. Large irregular necrotic ulcers developed on both calves, healing slowly to leave scars.

April, 1971. Further ulceration of both calves occurred following a haemolytic crisis, and rapidly developing areas of suppuration appeared in the acne tissues of his face and back (Fig. 1), with some resemblance to pyoderma gangrenosum. A sudden left hemiparesis and hemianaesthesia developed, from which he recovered; it was assumed to be due to cortical vein thrombosis.

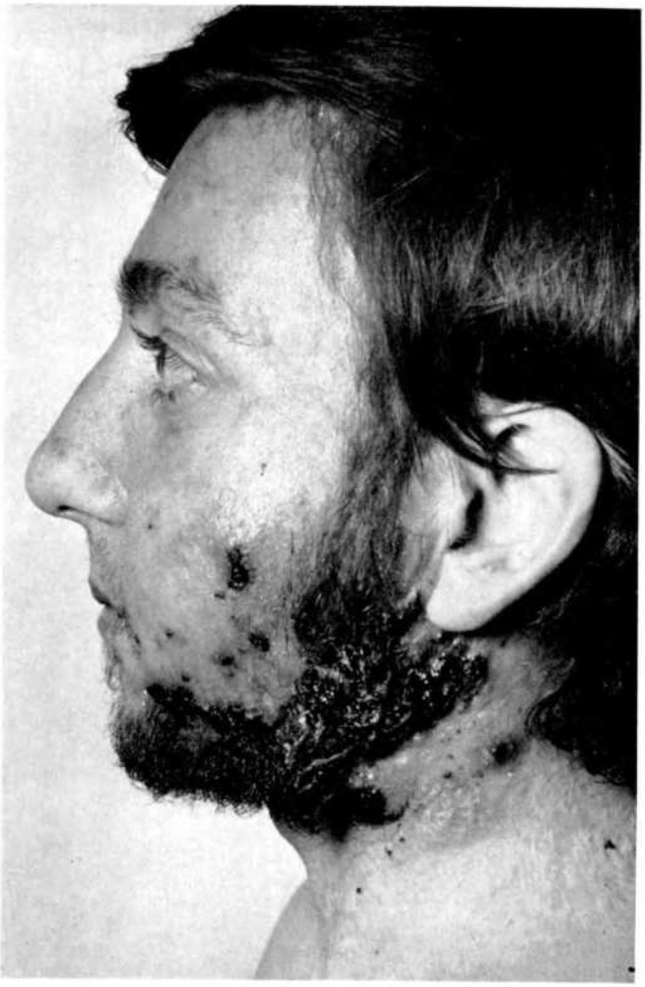

FIGURE 2. Ulcerative suppuration of face.

Two weeks later he developed abdominal pain with vomiting. Inferior veno-caval obstruction was confirmed by venography. He was anticoagulated with Warfarin. The leg ulcers improved slowly. He was discharged home on anticoagulants.

Between January and July, 1972, three blood transfusions were given for severe anaemia. There was recurrent ulceration of the calves. Further severe suppurative ulcers again developed in the acne on the shoulders and face (Fig. 2). These began with nodular haemorrhagic areas, spreading rapidly and ulcerating, healing slowly with marked scarring. The lesions were self-limiting and were unaffected in their course by topical steroids or systemic antibiotics.

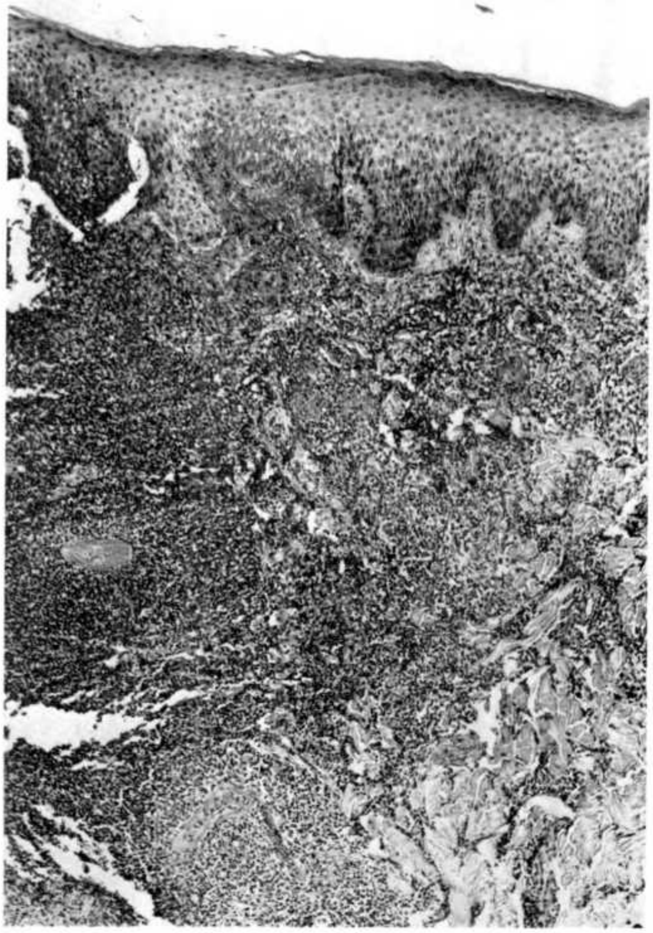

FIGURE 3. Biopsy of the shoulder showing suppurative and perivascular infiltration with fibrin deposits in small blood vessels (× 63).

August, 1972. Left hemiplegia due to cortical vein thrombosis.

March, 1973. Blood transfusion; anticoagulants continued.

April, 1973. Recurrent ulcerative suppuration on acne affected areas of cheeks and shoulders, but healing within a few weeks.

Investigations 1970–1973. Blood. Hb 9g/100 ml (1970), 10·3 g/100 ml (1973). WBC 5000/mm^3

(1970), 4700/mm^3 (1972), 2800/mm^3 (1973). Platelets 61,000/mm^3 (1970), 62,000/mm^3 (1973) Reticulocytes 12% (1970). Blood film—polychromasia and anisocytosis. ESR 70 mm/1 h (Westergren). Acidified serum (Ham's) test positive. Serum haptoglobins 5 mg/100 ml (normal 15–275 mg/100 ml). Moderate haemosiderinuria (1·5 mg in 24 h). *Liver function tests* (1973). Total protein 85 g/l (normal 60–80 g/l). Albumin 40 g/l (normal 35–48 g/l). Bilirubin 29 μmol/l (normal up to 17 μmol/l). SGOT 25 IU/l (normal 5–35 IU/l). SGPT 21 IU/l (normal 5–28 IU/l). SHBD 840 IU/l (normal up to 360 IU/l). Alkaline phosphatase 125 IU/l (normal 10–40 IU/l).

Immunological investigations. Immunoglobulins normal. Direct Coombs test negative. Total serum complement level 720 units/ml (normal range). Polymorph function tests. (a) Phagocytosis; polymorph preparations ingested test particles normally, the average proporation of phagocytosing cells being 79% (normal limits 65–89%). (b) Nitro-blue tetrzaolium test; 9% of the patient's cells reduced this dyestuff (within normal limits). Mantoux test; strongly positive at 1:1000 dilution.

X-rays. Inferior venocavogram showed complete occlusion of the iliac veins and the inferior vena cava. The upper level of the occlusion was not demonstrated. Collateral circulation was seen on the anterior abdominal wall, lateral abdominal wall and retro-peritoneally, connecting with the azygos veins and the internal mammary veins.

IVP. Bilateral equal excretion was seen. No radiographic evidence of renal vein thrombosis was seen.

Organisms cultured from suppurations included beta haemolytic streptococcus (Lancefield Group C), *Staphylococcus aureus* (coagulase positive), *S. albus* (coagulase negative).

Histology (Fig. 3). Ulcer on shoulder; acute suppurative and perivascular inflammation and involvement of the small vessels with fibrin deposits.

Discussion. The three essential features here are PNH 'recurrent leg ulcerations' and acne complicated by suppurations resembling pyoderma gangrenosum. PNH may be preceded by a period of marrow aplasia or hypoplasia and pancytopenia, as we observed in our own patient (Dacie, 1967). Venous thrombosis is a frequent complication of PNH and thrombo-embolism is a major cause of death. Leg ulceration has been noted only when the patient has been severely anaemic, and anaemia, venous insufficiency and thrombosis in the legs may be contributory factors here. Conglobate acne dates from adolescence at the age of 15, but the type of suppuration with burrowing pyoderma is not typical of acne conglobata, nor is it associated with haemolytic crises or severe anaemia, and it is self-limiting and uninfluenced by treatment. Cutaneous haemorrhagic necrosis has been observed in patients receiving coumarin anti-coagulants (Meyler & Herxheimer, 1972), but in these cases, ulceration is preceded by erythema and petechiae. Our patient, however, continued to develop suppurative lesions during a short period in which he was not receiving Warfarin. The clinical findings and investigations provide no evidence for the commoner causes of pyoderma gangrenosum; it was decided not to subject the patient to further barium studies because of his poor general condition. Tests so far performed do not suggest an abnormality of cellular or humoral immunity. The clinical progression of the lesions from induration and erythema to ulceration, together with the histology, suggest that there may be a vascular aetiology, but whether vessel occlusion from fibrin deposition is due to a cutaneous vasculitis, is secondary to inflammation of neighbouring tissue, or has resulted from intravascular coagulation associated with PNH is not clear.

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(4) Pyoderma gangrenosum and ulcerative proctitis

DR P.J.A.HOLT AND DR E.WADDINGTON

Department of Dermatology, University Hospital of Wales, Cardiff

S.J.B., female, aged 75 years.

History. October, 1969, admitted to hospital with a history of diarrhoea and passing blood and slime per rectum. A diagnosis of ulcerative proctitis was made, and treatment with Predsol suppositories and salazopyrine was started. She became symptom free. In March, 1972, salazopyrine was discontinued. July, 1972. While on holiday abroad, she had a relapse of proctitis. She received no treatment until her return home a month later. August, 1972. She developed two 'boils', one on the left side of the vulva and the other in the left axilla. Both lesions broke down to form large ulcers.

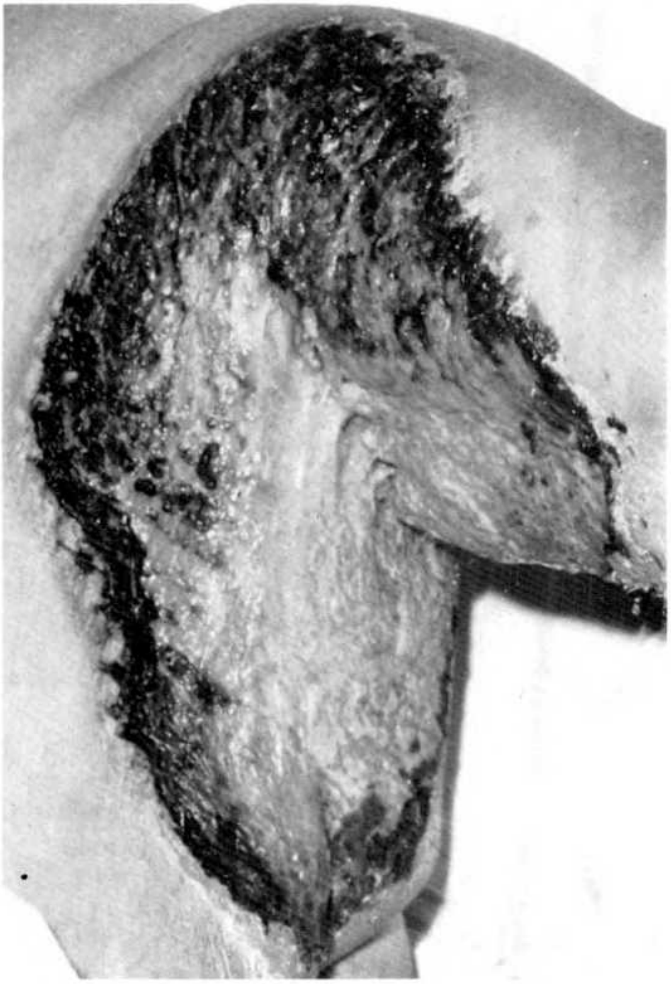

FIGURE 1. Left axilla showing the huge necrotic ulcer.

On examination (September, 1972). She had a generalized morbilliform toxic erythema. In the left axilla there was extensive and deep ulceration of the skin with an irregular margin and necrotic base spreading onto the medial aspect of the left upper arm and also onto the lateral aspect of the left breast (Fig. 1). The left labium majorum had a similar appearance. Abdominal examination; obese but not distended. Liver and spleen were not palpable. Sigmoidoscopy to 10 cm revealed a friable mucosa. Both legs were swollen from the foot to mid-calf with pitting oedema. Examination of the cardiovascular, respiratory and central nervous systems revealed no abnormality.

Investigations. Hb 11·1 g/100 ml. WBC 9700/mm^3. Platelets 220,000/mm^3. Blood film normal. ESR 95 mm/1 h (Westergren). Blood urea and electrolytes normal. Total protein 56 g/l (normal 60–80 g/l). Albumin 32 g/l (normal 39–48 g/l). Liver function tests; normal. Immunoglobulins; IgG 10·5 g/l (normal 4·5–16 g/l), IgA 4·80 g/l (normal 1·2–4·0 g/l), IgM 1·22 g/l (normal 0·45–1·8 g/l). Antinuclear factor test negative. ASO titre less than 50 units. Initial cultures from areas of ulceration were sterile. *Histology of rectal mucosa.* Compatible with ulcerative proctitis (Fig. 2). *Chest X-ray.* The heart size was normal, lung fields were clear. *Barium enema.* Diverticular disease of the transverse, descending and sigmoid colon. Colon and caecum were otherwise normal.

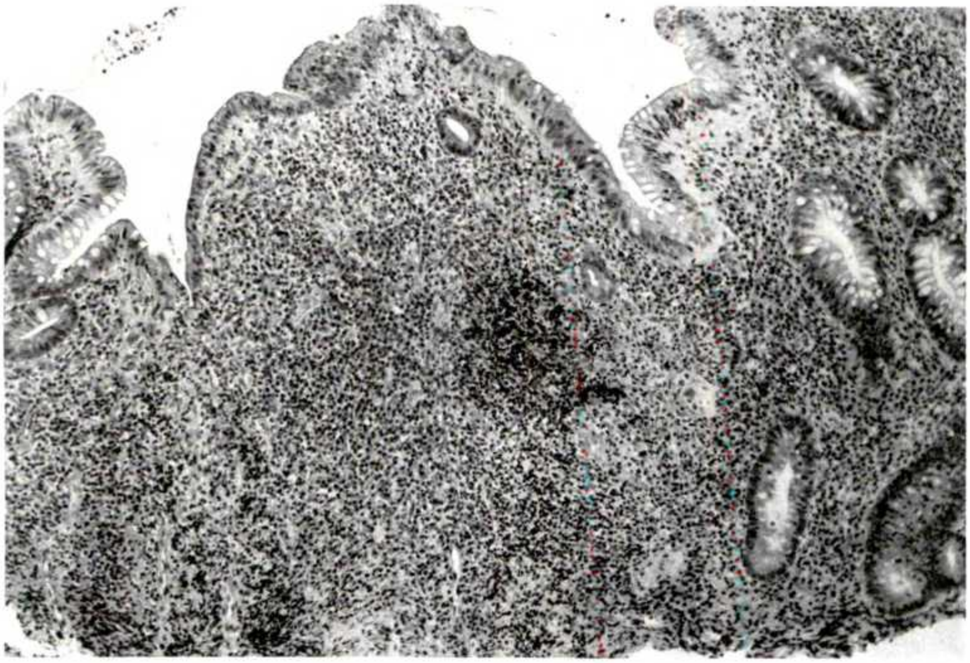

FIGURE 2. Rectal biopsy, showing acute inflammation (× 88).

Treatment and progress. She was treated with salazopyrine 8 g daily, prednisone 80 mg daily, and the areas of pyoderma were treated locally by irrigating with warm saline and later with half strength Eusol. 7 days after admission, she became severely ill and moribund. A diagnosis of congestive cardiac failure and peritonitis secondary to perforation of the bowel was made. Prednisone was reduced to 40 mg daily and she received digoxin, diuretics, potassium supplements and intravenous carbenicillin and gentamycin. 1 week later her general condition had begun to improve. During the next few months, the proctitis cleared and the ulcers healed. Prednisone was stopped in January, 1973. In

March, 1973, she was discharged home on salazopyrine 2 g daily. In April, 1973, she was readmitted with a recurrence of necrotic ulceration on the right lower abdomen. She had diarrhoea, but no blood or mucus.

Discussion. Pyoderma gangrenosum is not a common dermatological complication of ulcerative colitis. Goligher *et al.* (1968) found that only thirteen of 465 (3%) patients with ulcerative colitis developed pyoderma at some stage of their illness, and Johnson & Wilson (1969) reported only seven cases among 450 patients (1·6%).

Sigmoidoscopy and barium enema examination in our patient suggests that the bowel disease is limited to the rectum. The pyoderma developed during the severe episode of ulcerative proctitis, but whether or not more prompt control of the proctitis would have prevented the development or delayed progression of the pyoderma is not clear. Johnson & Wilson (1969) stressed that the onset of skin ulceration did not necessarily coincide with the exacerbation of ulcerative colitis, and that there was no parallel between the extent in severity of colonic involvement and severity of the pyoderma, but Goligher *et al.* (1968) concluded, from their series, that those patients who developed pyoderma usually did so during a severe attack of colitis.

In our patient, the pyoderma healed very slowly, and no improvement was seen until several weeks after the symptoms of proctitis had subsided. Delay in healing is not uncommon, and Edwards & Truelove (1964) reported cases of pyoderma which persisted for years, even when the symptoms of colitis were absent or slight.

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(5) Chronic repetitive suppuration of the skin associated with a serum factor affecting bactericidal capacity of polymorphonuclear leukocytes

DR G.A.HODGSON AND DR E.M.THOMPSON

University Hospital of Wales, Cardiff

A.M., female, aged 15 years.

History. Since 6 months of age she has had recurrent ulcerative scarring suppuration of the skin associated with persistent anaemia, polymorphonuclear leukocytosis, hepatosplenomegaly, chest infections and some stunting of growth. The suppuration begins either spontaneously or after trauma, (e.g. venous cut-down of the left leg (1965), ACTH injection (1971)) as raised haemorrhagic nodules which rapidly develop into large raw necrotic areas leaving massive scars. The gums have shown a hyperplastic gingivitis with periodontal hyperplasia and loosening of teeth. A massive ulcer of the left arm in 1965 was followed by a *Staphylococcus albus* septicaemia and hemiplegia from a cerebral abscess or embolus. Childhood viral infections (chickenpox and measles) and anti-tetanus immunization have been tolerated.

Family history. One sibling brother died at 18 months, of staphylococcal skin infection. One brother (aged 15) living, with no susceptibility to infections. Father (aged 38); normal immunoglobulins. Mother; hypergammaglobulinaemia 200 mg/100 ml.

Examination. Ulcerative suppuration of legs with scarring (Fig. 1), also on arms (Fig. 2), and hyperplastic gingivitis (Fig. 3).

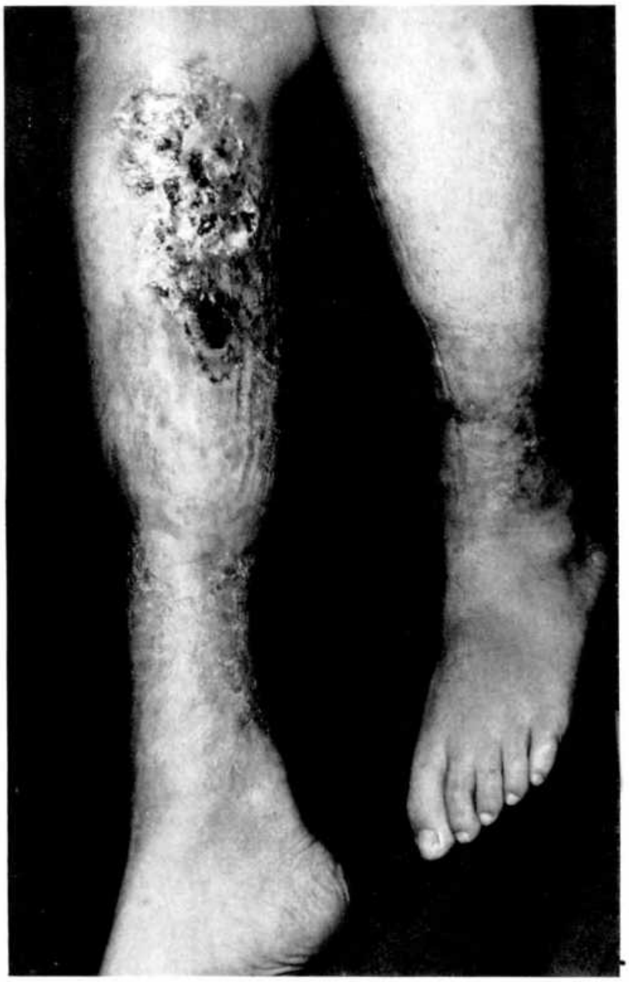

FIGURE 1. Typical suppuration of leg.

Investigations. *Blood*. Hb 77% (11·4 mg/100 ml). WBC 23,000/mm^3. MCV 74 μm^3 (76–96 normal). MCHC 24% (30–35% normal). Immunoglobulins: IgG 3840 mg/100 ml; IgM 400 mg/100 ml; IgA 62 mg/100 ml. *Bacteriology*. Skin: *Staphylococcus aureus*, *Pseudomonas pyocyanea*, Proteus. *Biopsy*. No granuloma. Endarteritis and chronic inflammation.

Immunological tests (Dr E.M.T.). (a) Bactericidal capacity of polymorphonuclear leukocytes against *S. aureus* by method of Holmes *et al*. (1966). (1) Definite impairment of intracellular digestion of patient's leukocytes in presence of her own serum. (2) Patient's serum similar but less inhibitory effect on mother's cells. (3) No effect on cells from father, live sibling or control. (4) Parents and brother normal leukocyte function with pooled serum. (b) Lymphocyte transformation test with PHA; normal. (c) Adequate antibody response to tetanus toxoid. (d) Serum complement; normal.

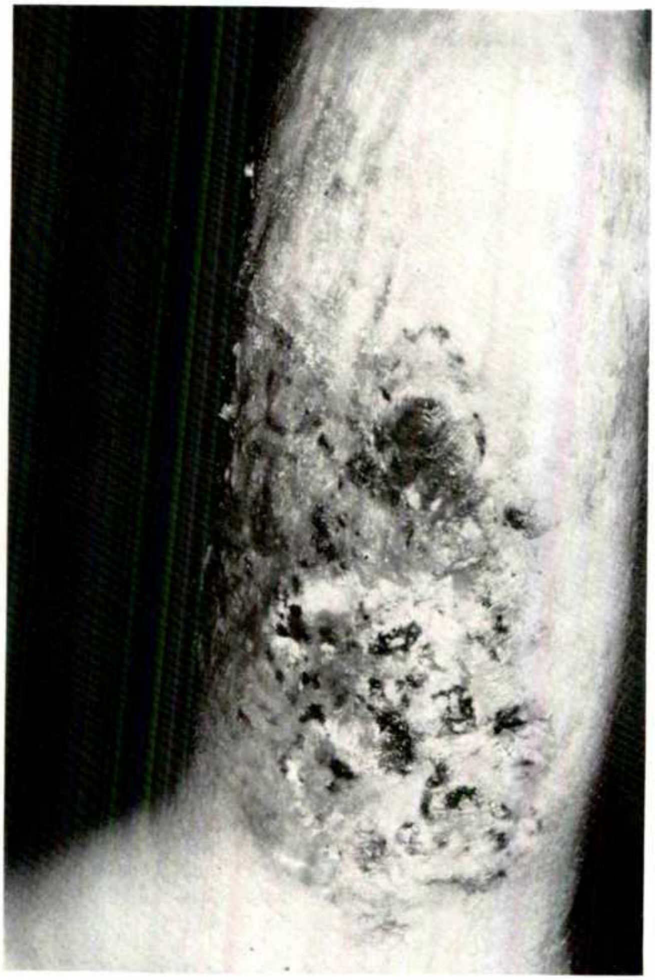

FIGURE 2. Typical suppuration of arm.

Treatment. No significant response over 6–7 years to gammaglobulin (1965), local and systemic antibiotics and steroids (1966–70).

Comment. The case has been shown at the Royal Society of Medicine (Hodgson & Thompson, 1972) and considered to be due to an unknown serum factor(?) circulating inhibitor or lack of substance required for normal leukocyte function. It differs from that described by Miller *et al.* (1968) of a child with some similarities whose serum contained a factor affecting phagocytosis but not intracellular digestion. In chronic granulomatous disease (Berendes, Bridges & Good, 1957; Bridges, Berendes &

Good, 1959; Thompson & Soothill, 1970), where the defect is also in the inability of the leukocyte to kill certain ingested organisms (Holmes *et al.*, 1966), visceral abscesses and granulomas occur and the suppurations are not restricted to the skin.

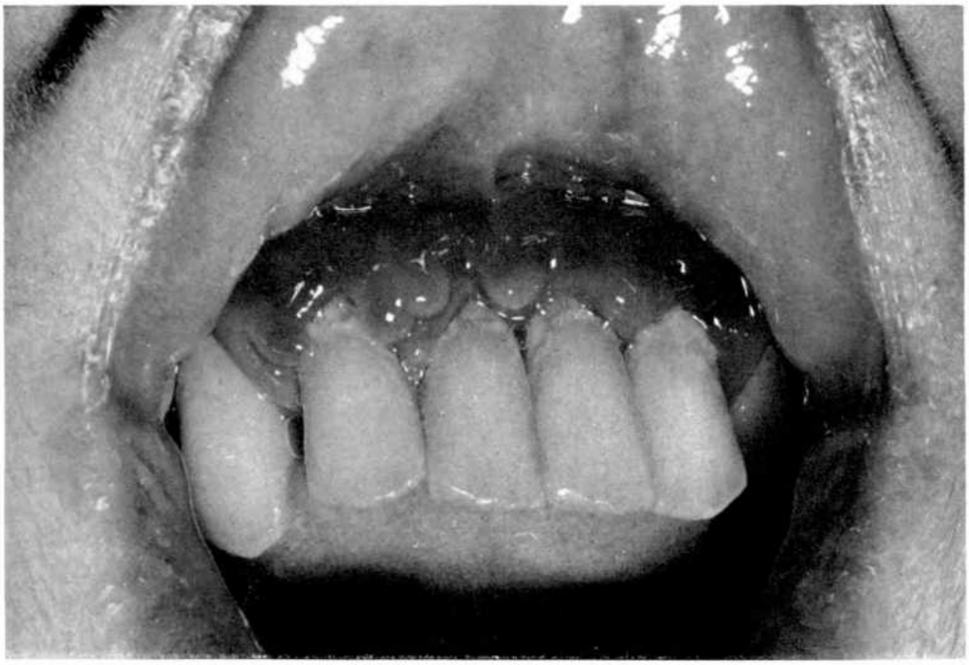

FIGURE 3. Appearance of gums.

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(6) Case for diagnosis. (?)Pyodermite végétante of Hallopeau

DR C. LONG

Department of Dermatology, University Hospital of Wales, Cardiff

R.N., male, aged 76 years.

History. In August, 1969, he developed symptomless bullae on the right sole. In January, 1970, he was seen in out-patients with vesicles, pustules and bullae on the left sole only, with the appearance of an infected dyshidrotic eczema. In February, 1970, bullae varying in size from 3 to 10 mm formed a line around the left instep and spread over the foot and lower two-fifths of the left leg leaving a denuded crusted area in its wake. The right foot and the leg were also affected (Fig. 1). In June, 1970, similar lesions developed on the finger tips and advanced over varying distances on the fingers. Some nails were shed. By February, 1971, all lesions had cleared. In June, 1971, he had a recurrence of lesions on hands and feet, and he has not been fully clear since.

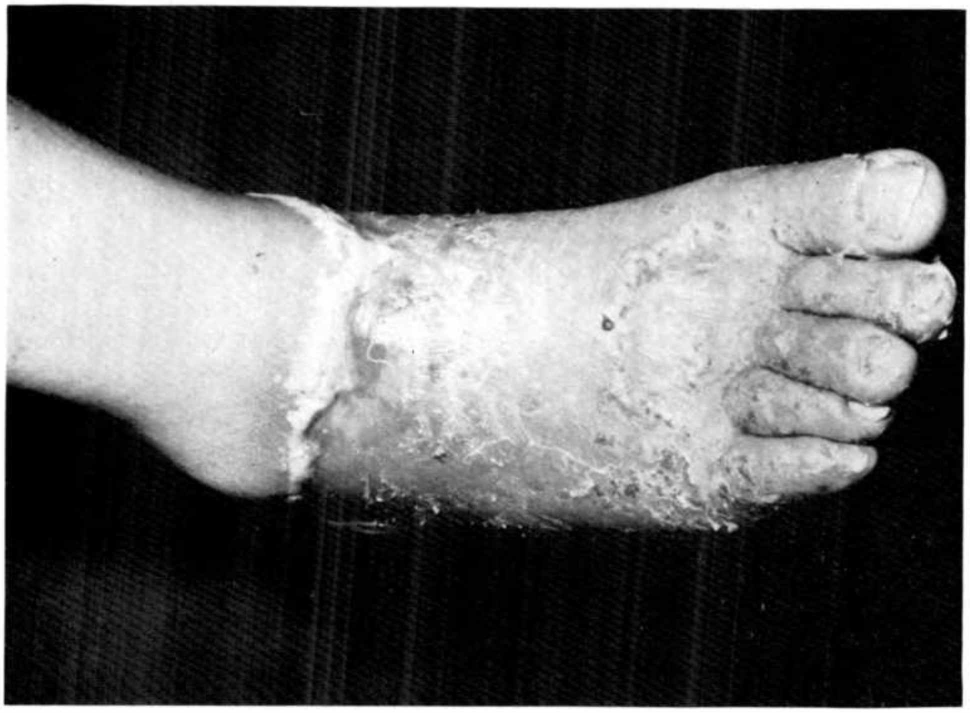

FIGURE 1. April, 1970. Right foot affected.

Investigations. Blood count, blood urea, LFTs, WR, VDRL, Reiter's CFT, viral studies (innoculation into suckling mice and electron microscopy), blood culture, serum calcium, skin scrapings cultured for fungus, all normal. ESR varied up to 70 mm/1 h.

Histology. (1) Behind advancing edge—acute inflammatory ulceration with no specific features other than intra-epidermal bullae. (2) Advancing edge—superficial pustule. Intra-epidermal oedema and erosion. Papillary corium is oedematous. Dilated capillaries with leukocyte infiltrate and some eosinophils. In the surface pustules there are many colonies of cocci which appear to be gram negative. (3) Before advancing edge. Pustule breaking through the surface in the manner of a small abscess.

Barium enema. Extensive diverticular disease of the sigmoid colon.

Serum electrophoresis. Slightly decreased albumin and slightly increased alpha-2-globulin.

Faecal occult blood. Positive × 2.

Chest X-ray. Hypertrophic left ventricle. Dense and elongated aorta. Both costophrenic angles blurred.

Multiple swab for culture. Organisms varied—beta haemolytic streptococci, coliforms, proteus, *Staphylococcus aureus*, diphtheroids, *Pseudomonas pyocyanea*, *Candida albicans*.

Treatment. Multiple carious teeth were extracted and ampicillin, tetracycline, nystatin and griseofulvin were administered orally; genticin, nystatin, Gentian Violet, betadine, propaderm, betnovate and triadcortyl topically.

Comment. The condition fluctuated irrespective of therapy. The axillae and groins were not involved as is usual in pyodermite végétante of Hallopeau. Neither were there any scars or erosions on the mucous membranes.

Surgical treatment of the diverticular disease of the colon was not considered necessary as the colitis caused very few symptoms, and because there is no evidence that removal of a diseased colon influences the skin condition (Vickers & Guerrier, 1971).

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(7) **Oral mucosal neuroma–medullary thyroid carcinoma syndrome**

MR D. M. WALKER

Department of Oral Medicine, The Dental School, University Hospital of Wales, Cardiff

J.S., male, aged 21 years.

History. February 1969: total thyroidectomy for 'papillary' carcinoma of thyroid (by Mr J.S.H. Wade). October 1971: attended Dental Hospital when nodules on dorsum of tongue, present 'since childhood', were reassessed and a diagnosis of oral mucosal neuroma–medullary thyroid carcinoma syndrome was made and confirmed by biopsy of tongue, and re-examination of 'papillary' thyroid carcinoma sections.

Family history. Paternal grandfather—'toxic goitre'. No mucosal swellings or thyroid malignancy in relatives.

On examination. No signs of thyroid tumour recurrence. Typical facies with full, pigmented, protuberant lips. Multiple firm sessile mucosal nodules present on anterior dorsum tongue (Fig. 1). Soft polyp behind each commissure. Pigmented naevus right thigh. Bilateral pes cavus.

Investigations. Blood. Random serum calcitonin 100 ng/ml (significantly above normal value: 200 pg/ml). *Urinary* catecholamine derivatives raised but not diagnostic (VMA 9·2 mg/24 h, total metadrenaline MA 0·94 mg/24 h). *Biopsy.* Mucosal nodule from dorsum of tongue (D678/71) (Fig. 2). Histology proved them to be true neuromas consisting mainly of intertwined bundles of nerve fibres.

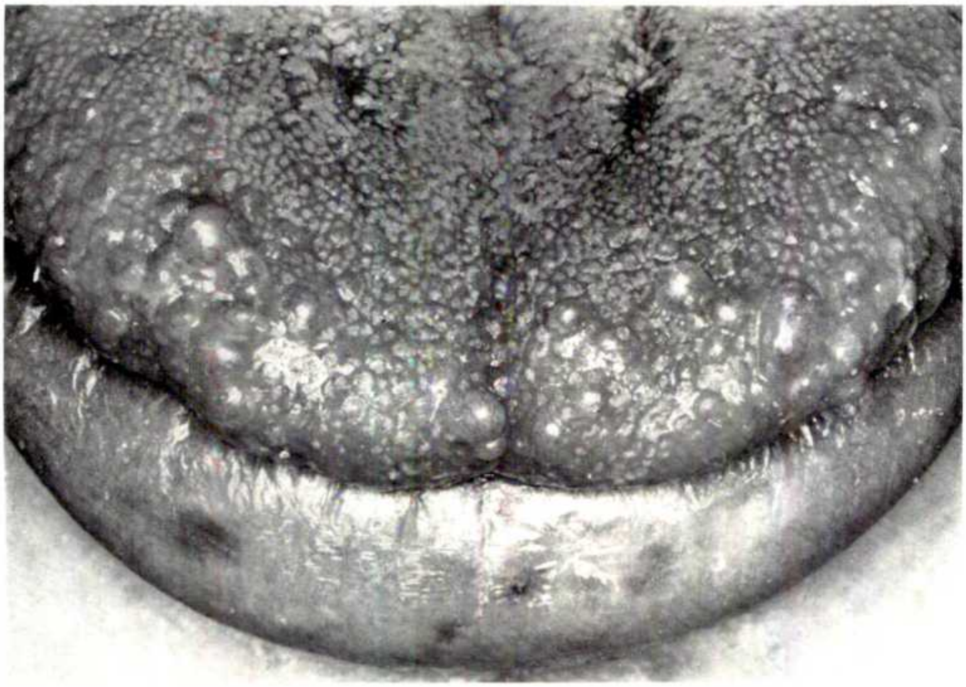

FIGURE 1. Multiple neuromas on anterior dorsum of tongue.

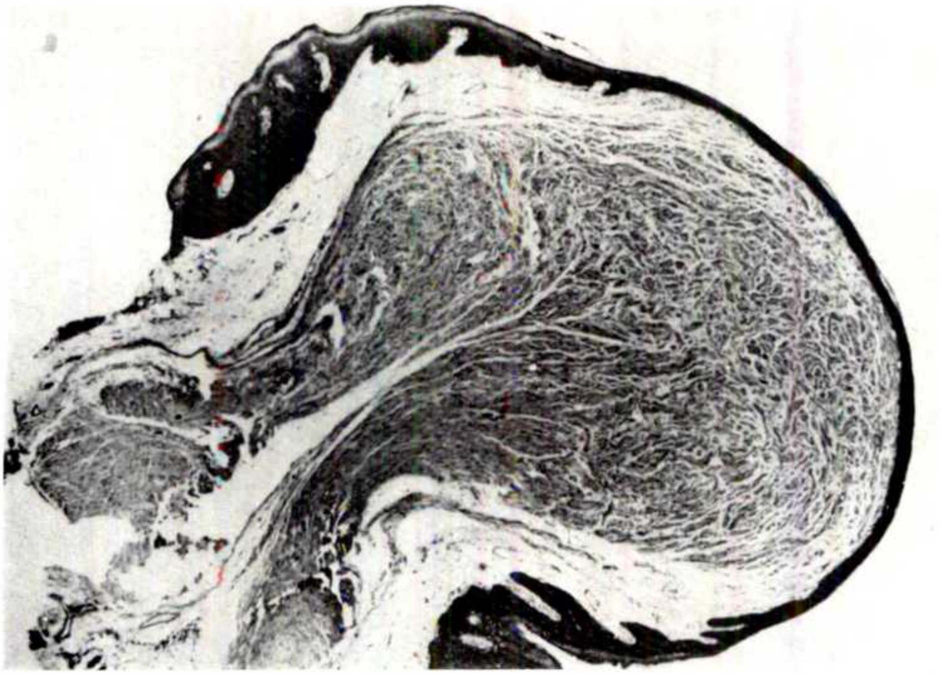

FIGURE 2. Section of one of the nodules from dorsum of tongue. Histology confirms them to be true neuromas, consisting mainly of intertwined bundles of nerve fiibres (silver, × 5·6).

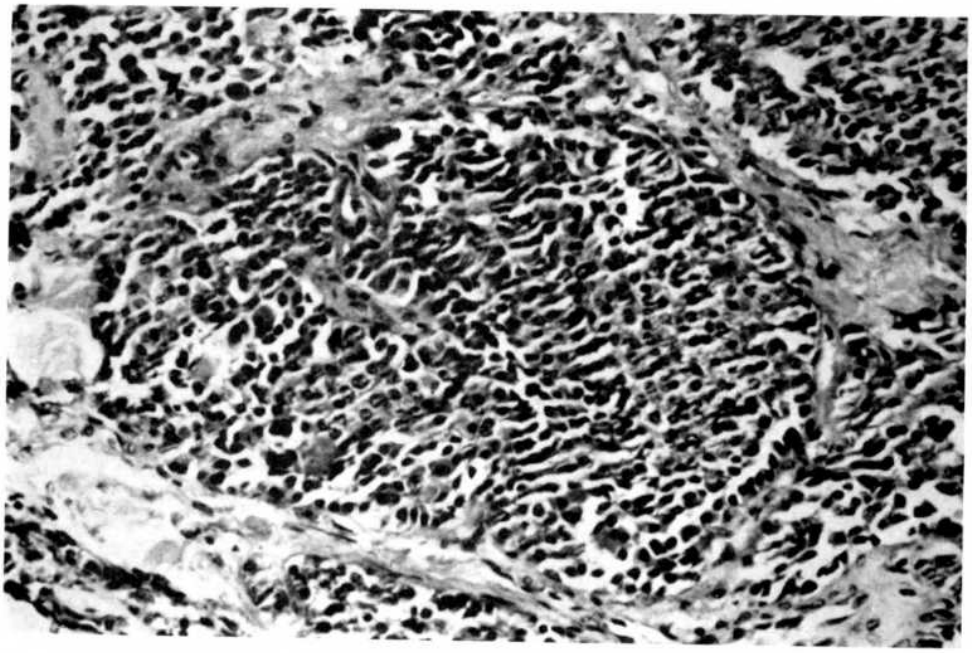

FIGURE 3. Histological appearance of the medullary thyroid carcinoma, with typical sheets of rounded cells, separated into islands by stroma (H & E, × 32).

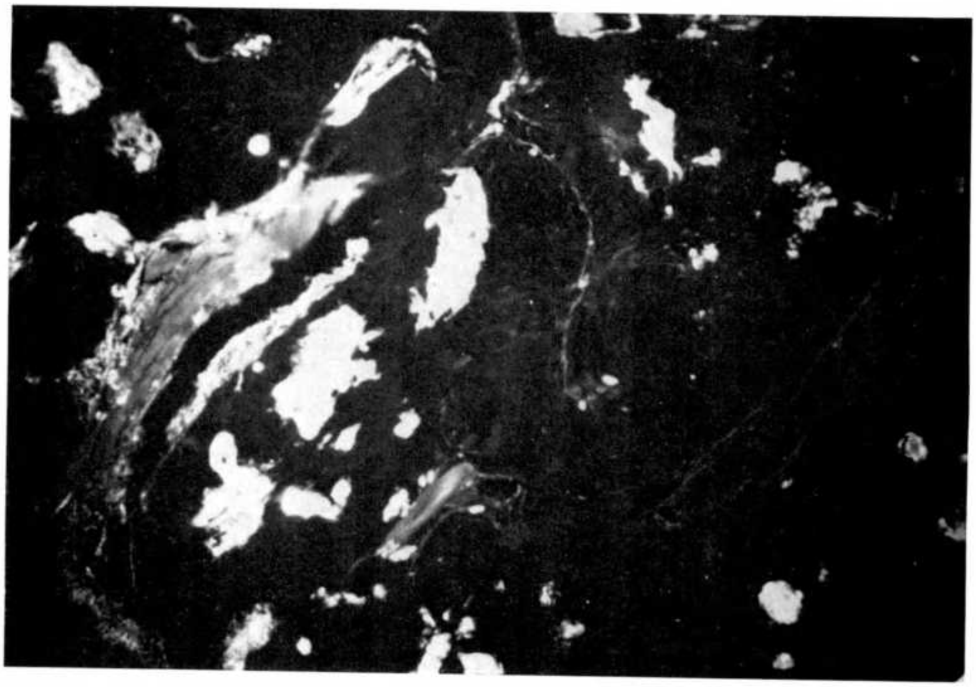

FIGURE 4. Section of the medullary thyroid carcinoma, the amyloid material fluoresces under ultraviolet light (Thioflavin-T, × 22).

Total thyroidectomy specimen (290/69). The so called 'papillary' tumour was in fact a medullary thyroid carcinoma, consisting of sheet-like masses of rounded cells (Fig. 3) separated into islands of variable size by the irregular trabecular stroma containing identifiable amyloid (Fig. 4).

Progress. Patient clinically free from tumour recurrence 3 years after thyroidectomy. However, now complains of 'nerves in stomach' and excessive sweating.

Comment. (1) J.S.'s prognosis worsened by changing the diagnosis from papillary to medullary carcinoma. The para-follicular 'C' cells of this tumour secrete calcitonin; hence the raised random serum calcitonin is evidence for presence of previously unsuspected residual tumour. (2) J.S. more likely to be a patient with a genetically determined (autosomal dominant) rather than a sporadic thyroid carcinoma. Genetic counselling will have to be considered. (3) Other endocrine tumours, e.g. phaeochromocytomas, can occur in this syndrome. J.S.'s new symptoms of sweating and 'nerves in stomach', together with raised urinary catecholamines, are suggestive of occult phaeochromocytomas. (4) As in this case, the oral mucosal neuromas, arranged in a pathognomonic distribution, appear in the first few years of life. They antedate the potentially fatal thyroid malignancy by up to 10 years, hence the importance of awareness of the syndrome when confronted by multiple oral mucosal neuromas.

A full report of this case, with a review of the literature, appeared in the *British Journal of Dermatology* (1973) **88**, 599.

(8) Dermatomyositis and carcinoma of the rectum (resected)

DR A. NAVARATNAM AND DR E. WADDINGTON

Department of Dermatology, University Hospital of Wales, Cardiff

Mrs N.S., aged 60 years. Housewife.

History. In 1954, she suddenly developed swelling of the face, neck and arms associated with severe muscle weakness and tenderness. She was unable to sit up and had dysphagia.

Examination (1954). There was livid heliotrope discolouration of the face, neck and scalp, with gross oedema of the eyelids, cheeks and arms. There was fixed linear erythema over the dorsa of the hands and fingers (Fig. 1), and patchy erythema on the elbows, knees and buttocks. Severe muscle weakness, most marked in the shoulder girdle and quadriceps.

Investigations (1954). Serum albumin 2·1 mg/100 ml. Globulin 4·3 mg/100 ml. Creatine output of urine varied from 800 to 1050 mg in 24 h. ECG normal.

Skin biopsy. The biopsy shows epidermal and dermal changes. There are in the dermis numerous dilated vessels extending occasionally up to the epidermis. There is in the upper dermis a rather diffuse infiltrate of lymphocytic and polymorphonuclear type which is involving the lower part of the epidermis. The epidermis shows some intracellular oedema and disruption of the basal layer with a small zone of acantholysis. Most of the epidermis shows some hyperplasia and definite hyperkeratosis.

Muscle biopsy. Muscle biopsy shows focal areas of lymphocytic infiltrate around vascular bundles and also involving the muscle fibres. There are occasional areas of degenerating muscle with some sarcolemmal cell proliferation. These features are consistent with dermatomyositis.

Course. She was treated with cortisone and later prednisone (10–25 mg daily). There was gradual increase in muscle power, but she developed severe diffuse alopecia of the scalp.

1958. Hysterectomy performed for menorrhagia. No evidence of malignancy.

Repeated admissions from 1958 to 1963 to exclude malignancy proved negative.

1960. X-ray of spine showed osteoporosis and partial collapse of T1, L2 and L3 vertebrae.

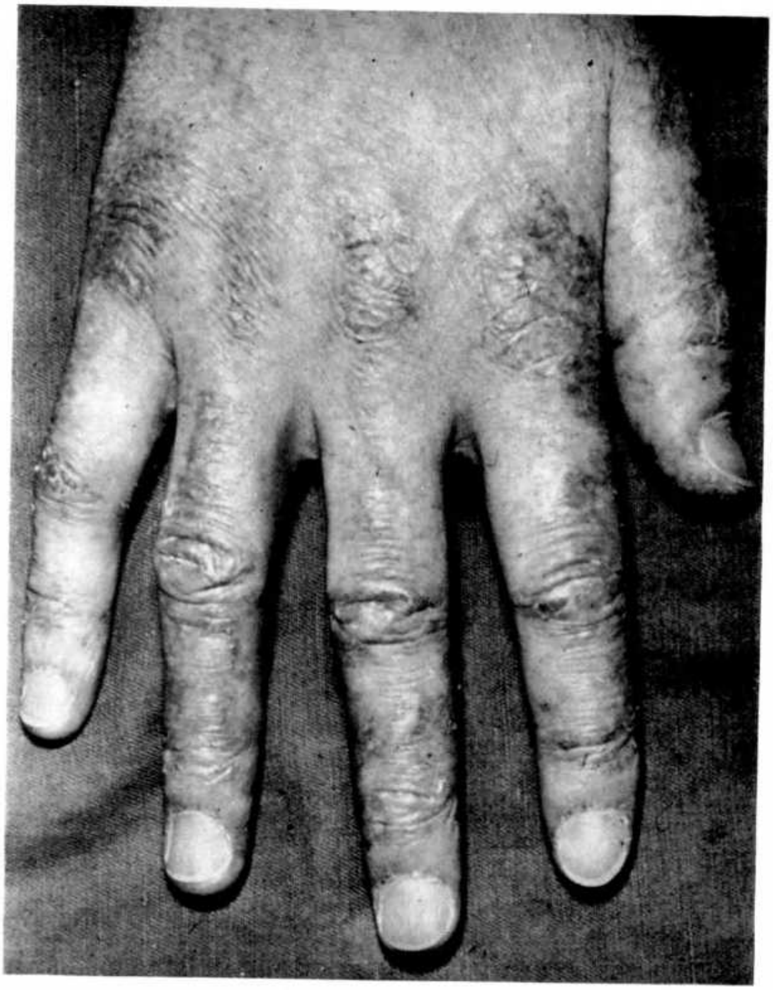

FIGURE 1. Dermatomyositis. Fixed linear scaly erythema over the dorsum of the fingers.

1963. Onset of rectal bleeding.

1964. Abdomino-perineal resection of carcinoma of the rectum. *Histology.* Grade II adenocarinoma with no invasion of the serosa or adjacent lymph glands.

1973. During the last 9 years she has been able to do her housework. The urine creatine has varied from 16 to 36 mg per 24 h. She has been maintained on prednisone 10 mg daily. *Examination.* There is diffuse erythema with areas of poikiloderma on the scalp, forehead, face and neck. There are indurated lichenified plaques on the extensor aspects of the elbows, thighs, left arm and nape of the neck (Fig. 2). Gross wasting of the limb muscles. *Histology* of an indurated plaque (Fig. 3). Skin biopsy showing a zone of epithelial hyperplasia and hyperkeratosis. At the dermo-epidermal junction in this area there is a homogeneous pink band of material which in part is positive for fibrin, collagen, and also mucin. Deep to this zone are numerous dilated capillaries and a minimal inflammatory round cell infiltrate.

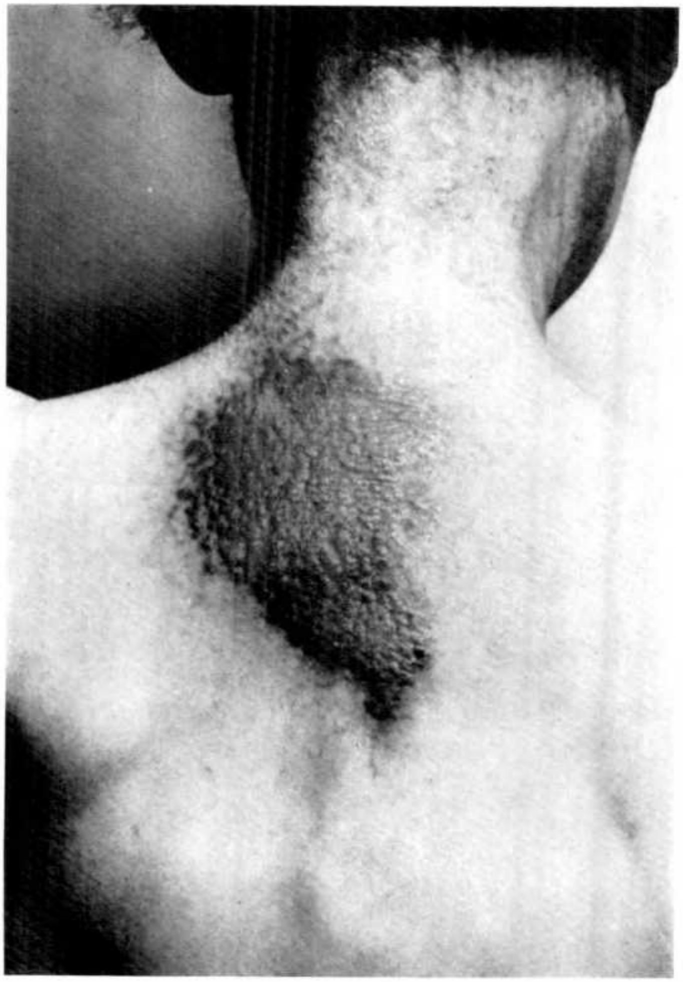

FIGURE 2. 18 years later. Poikiloderma on the neck. Livid indurated plaque on the back.

Comment. An unusual feature here is that the dermatomyositis preceded the detection of the neoplasm by over 9 years. Arundel, Wilkinson & Haserick (1960), in a 20-year survey of patients with dermatomyositis, estimated the average time interval to be about 6 months. The longest interval in their series was 3 years. There has been little change in the dermatomyositis following the successful removal of the rectal carcinoma. Her most distressing symptom is severe burning and irritation of the scalp which becomes unbearable if the dose of prednisone is reduced below 10 mg daily.

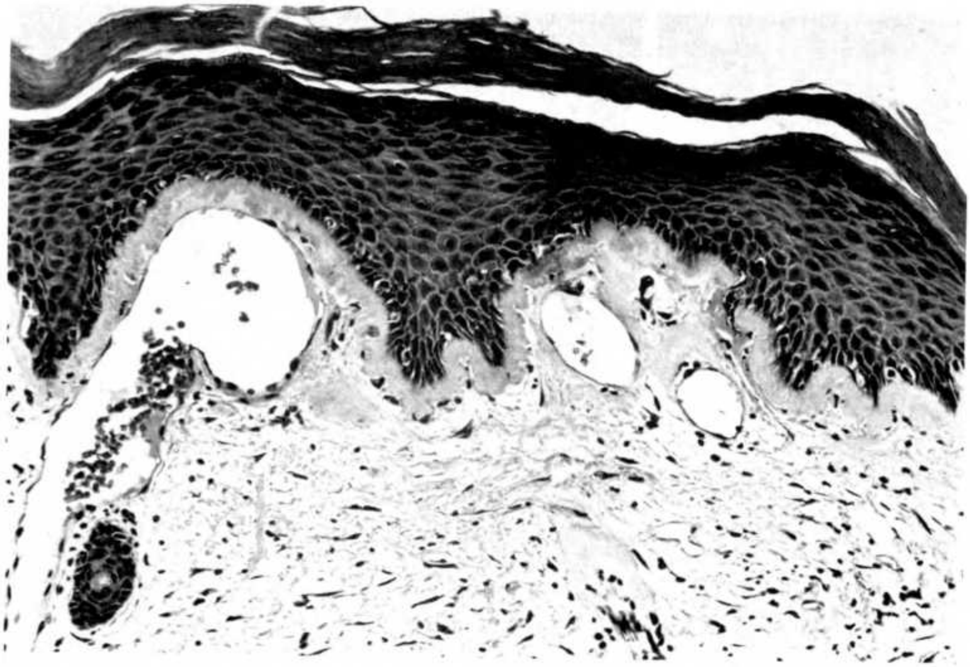

FIGURE 3. Histology of the indurated plaque. Note the homogeneous band of material and dilated capillaries (× 123).

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(9) Acanthosis nigricans with carcinoma of the stomach

DR A. NAVARATNAM AND DR G. A. HODGSON

Department of Dermatology, University Hospital of Wales, Cardiff

T.L., male, aged 63 years.

History. August, 1972, admitted with melaena and loss of weight. No previous history of indigestion, abdominal pain or vomiting. 3 weeks before admission noticed darkening of the skin of the axillae, groins and sides of the neck, with non-irritable small nodules in the axillae and groins, and multiple 'warts' on the limbs. November, 1971, haematemesis; barium meal then apparently normal.

Family history. Father died of carcinoma of the rectum. Mother died of carcinoma—site not definite. No relevant drug history.

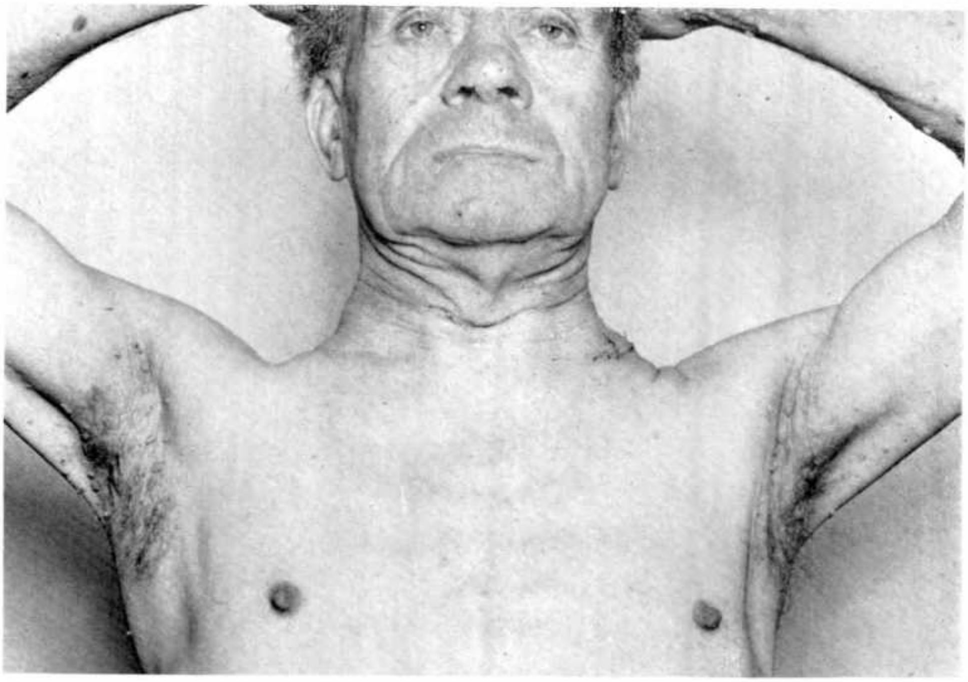

FIGURE 1. Slaty grey thickened skin of axilla with basal cell papillomata.

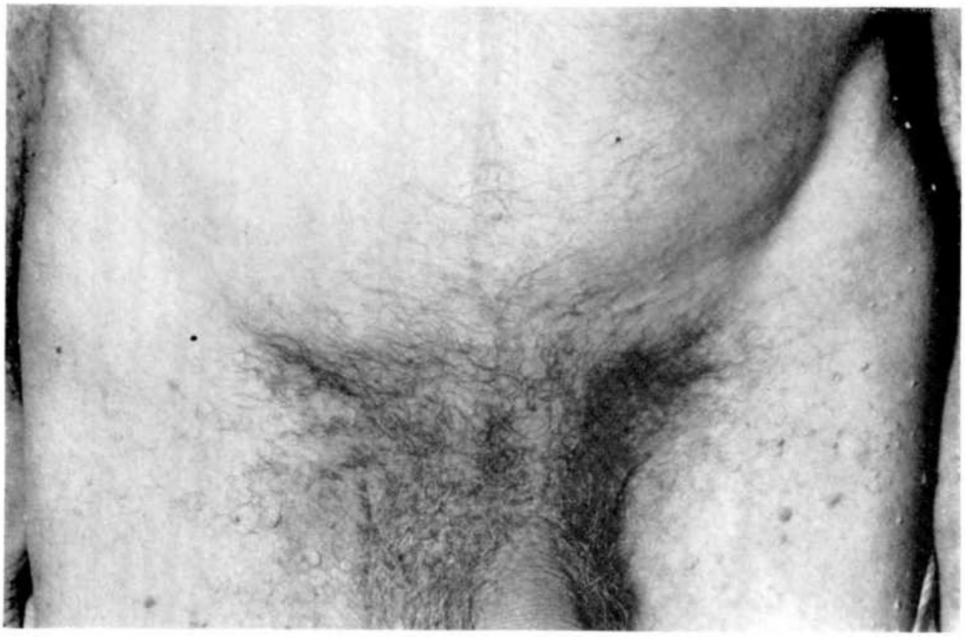

FIGURE 2. Slaty grey pigmentation of the groin with basal cell papillomata.

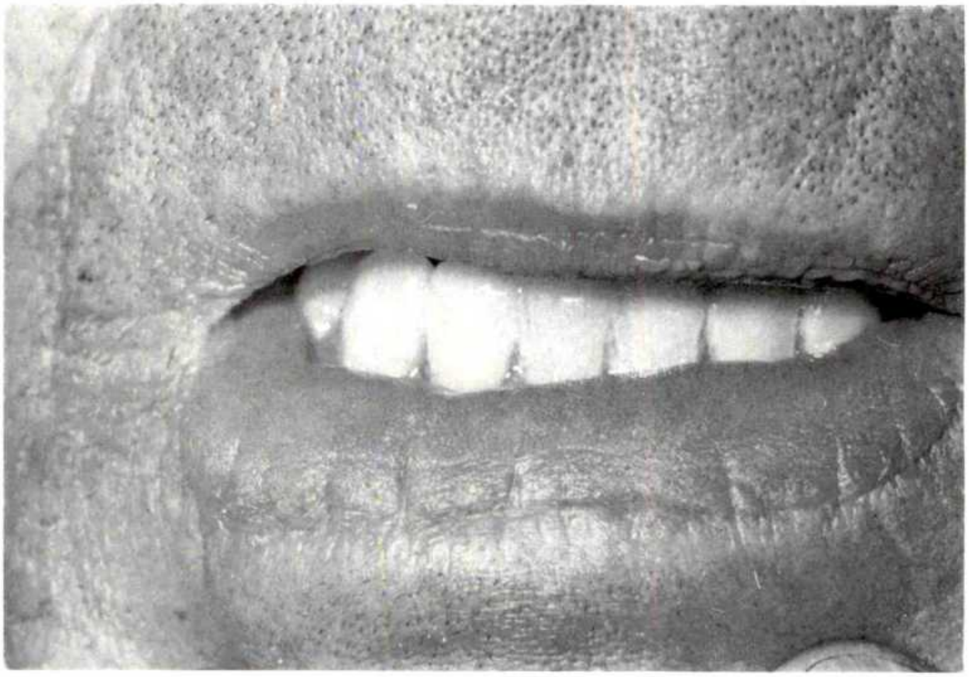

FIGURE 3. Warty mamillated appearance of the lower lip.

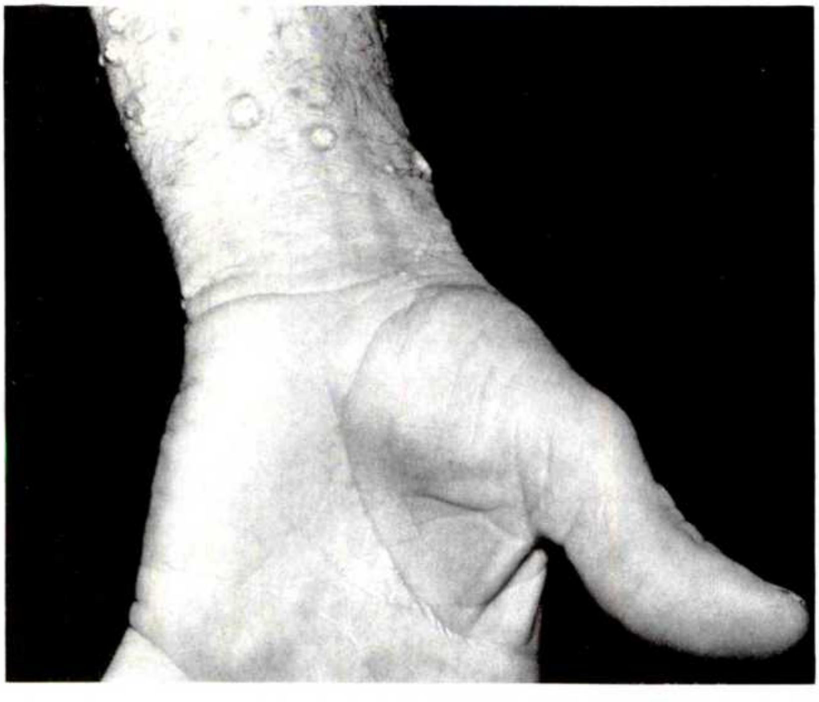

FIGURE 4. Several 'warts' on the wrist.

On examination. Clinically anaemic. *Alimentary system.* No physical signs of neoplasm. There was a firm enlarged lymph node in the left supra-clavicular fossa. Black tarry stool on rectal examination. *Skin.* The axillae (Fig. 1), sides and back of the neck, groins (Fig. 2) and perianal region, were slaty grey in colour, and velvety. The axillae and groins showed prominent folds and papillomatous elevations, like basal cell papillomata. The mucosa of the lower lip was mamillated (Fig. 3). Numerous warty nodules on the forearms (Fig. 4), dorsa of the hands and legs, resembling viral warts.

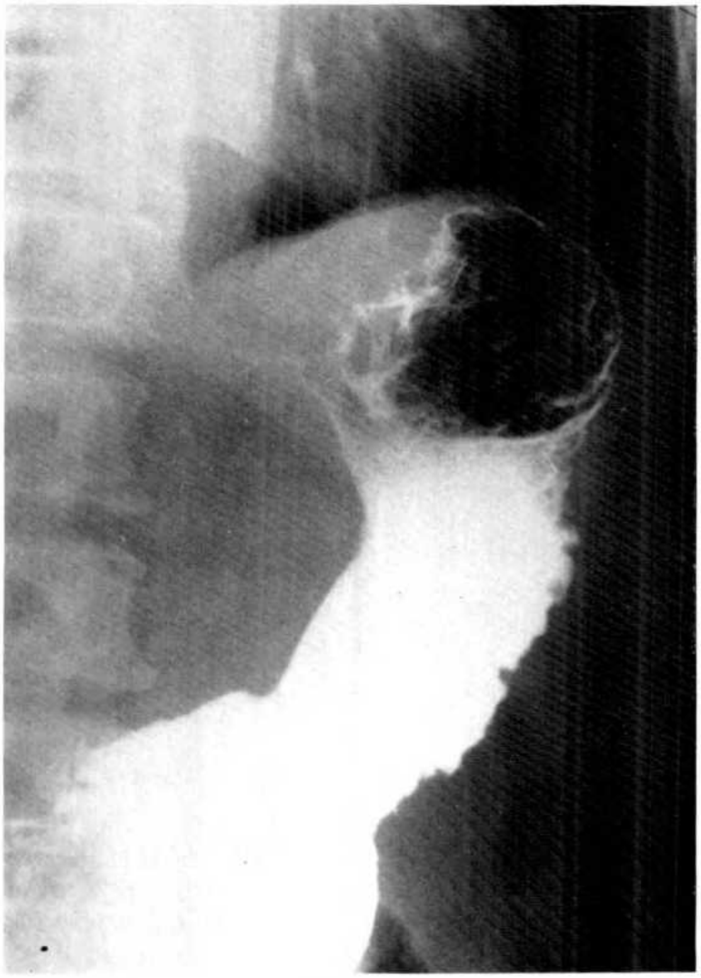

FIGURE 5. Barium meal irregularity of the fundus and greater curvature of the stomach.

Investigations. Hb on admission 11·1 g/100 ml. White cell count 4000/mm^3, with a normal differential. ESR 50 mm/1 hr. Liver function tests normal.

X-rays. Screening. Elevation of the right hemi-diaphragm with mass in the right hilum. *Tomograms* showed a right paratracheal and a right hilar mass, probably glandular, and presumably secondary deposits. *Barium meal.* The stomach shape was abnormal and irregular, (?)infiltrated with neoplasm, especially around the greater curve and on the fundus (Fig. 5).

Histology. Skin biopsy. 'Warts'; features are consistent with a squamous papilloma (Fig. 6). *Axillary skin.* Features are consistent with acanthosis nigricans. *Lymph node.* Almost entirely replaced by poorly differentiated adenocarcinoma, with tumour cells in large lymphatics surrounding the node. The appearances are consistent with a primary gastric carcinoma.

Course and treatment. No surgical intervention. On cyclophosphamide the patient's appetite improved, he is gaining weight, and the skin lesions are regressing.

Comment. Acanthosis nigricans can be a skin marker of neoplasia. It is not a common condition, but Curth (1952) has reviewed 350 cases since the condition was first described by Pollitzer (1890). The wart-like lesions on the forearms and lower limbs are clinically similar to virus warts, resembling the clinical variant reported by Jablonska, Kozminska & Chorzelski (1965), but the histology is that of squamous papillomata, and viral studies on the excised tissue were negative. It is interesting that this patient shows, in addition to acanthosis nigricans and basal cell papillomata, the sudden occurrence of numerous squamous cell papillomata, which have hitherto not been mentioned in the literature.

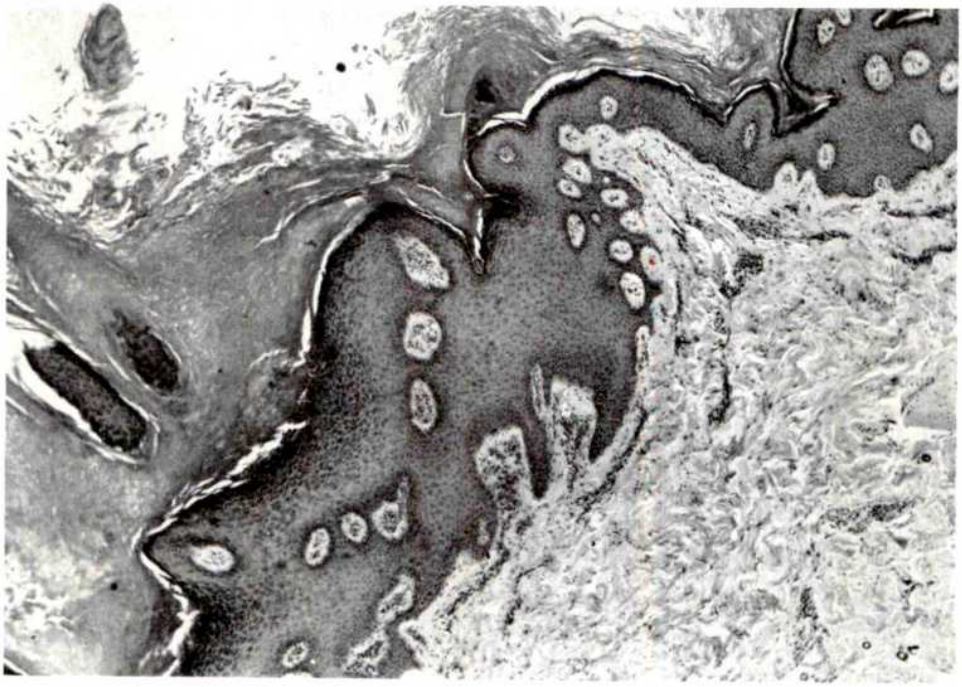

FIGURE 6. Histology of a 'wart'; features consistent with squamous cell papilloma (× 31).

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(10) Case for diagnosis

DR J.D.T.KILBY FOR DR R.R.M.HARMAN

Department of Dermatology, Bristol Royal Infirmary

Mrs D.B.A., aged 46 years. Housewife.

History. 8 years ago, the patient noticed a symptomless, black spot above her right ankle. Very gradually, similar lesions have appeared on her legs, and more recently, numerous bright red spots have developed on her trunk. 6 years ago, flat yellow lesions developed around her eyes.

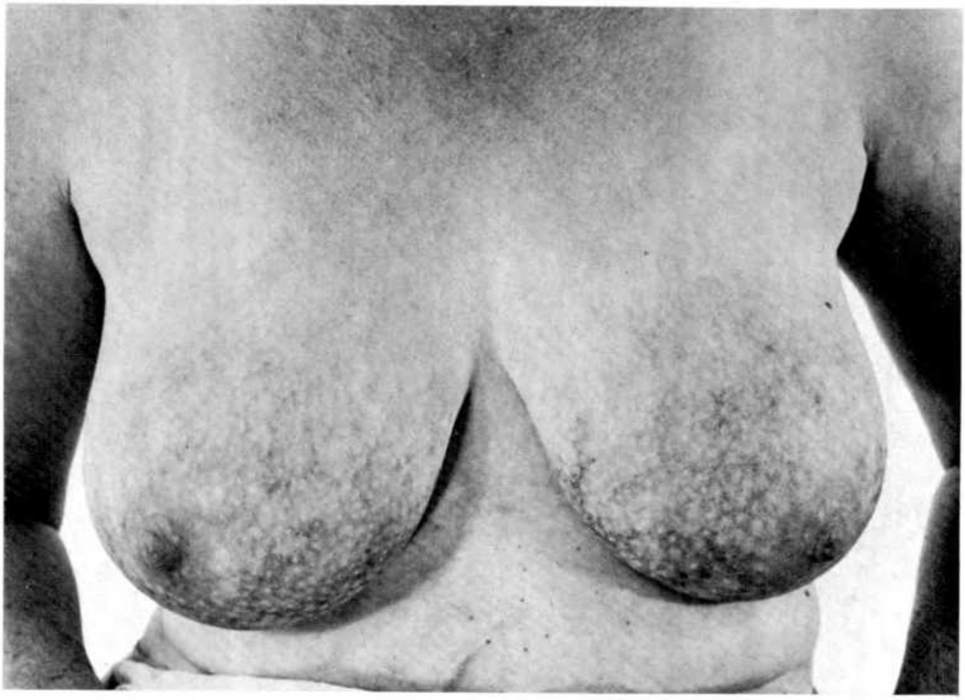

FIGURE 1. Mottled appearance on breast simulating poikiloderma.

Past history. Eczema as a child. Rash on neck 20 years ago; (?)diagnosis. Appendicectomy 1951. Pneumonia 1953. Cholecystectomy 1970. Since then has noticed colicky, lower abdominal pain, and irregular bowel habit. Sigmoidoscopy and barium enema 1971; normal. Drug eruption August 1971; two weeks following penicillin for insect bites.

Family history. Father (aged 69); left ventricular failure. Mother (aged 68); alive and well. Siblings; none. One son (aged 24); alive and well.

Examination. Blood pressure 160/100 mmHg. Xanthelasma palpebrarum. Mottled changes over the breasts, which at first appeared poikilodermatous. Campbell De Morgan's spots over trunk. Hyperkeratotic, black, angiomatous lesions over legs. Skin over shoulders and trunk; mottled appearance. Palpable, firm liver edge.

Investigations. ESR 14 mm/1 h (1967); 41 mm/1 h (12 October 1971); 46 mm/1 h (8 December 1972). WBC and differential; normal. Platelet count; normal.

Serum proteins. Levels and electrophoresis; normal (1971). Globulin 4·1–4·4 g/100 ml (December 1972).

Liver function tests: ↑aspartate aminotransferase 41 IU/100 ml (12 October 1971); 34 IU/100 ml (13 December 1972); ↑alkaline phosphatase 48 KA units/100 ml (12 October 1971) 60–65 KA units/100 ml (December 1972); serum bilirubin 0·6 mg/100 ml (December 1972).

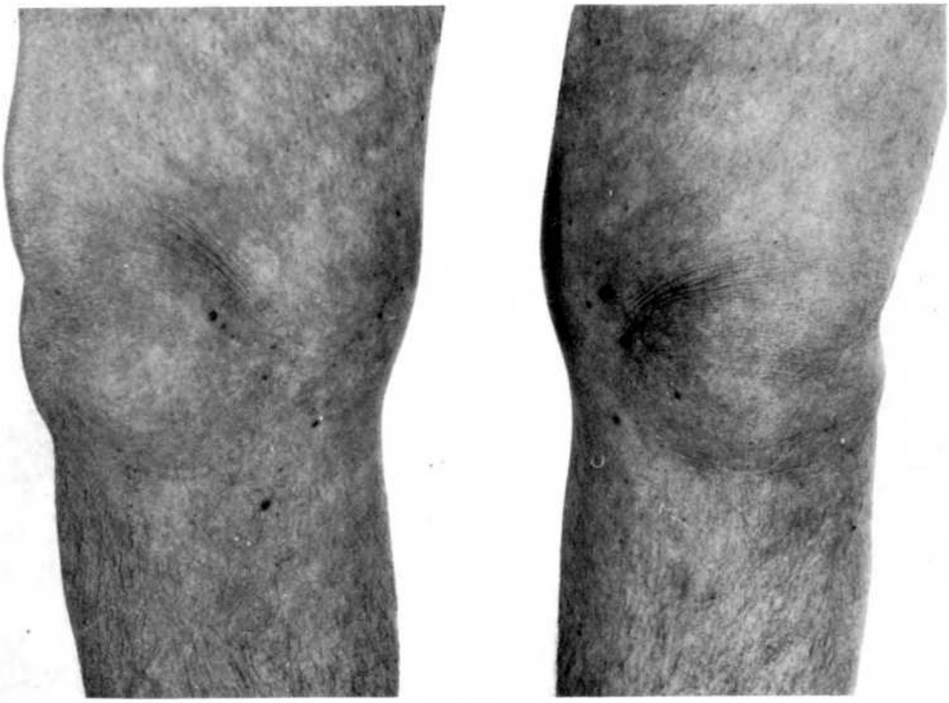

FIGURE 2. Telangiectasia and angiomatous lesions on legs.

↑5 Nucleotidase 119 IU/l (14 October 1971); 210 IU/l (December 1972).

↑Serum cholesterol 275 mg/100 ml (December 1971); 304 mg/100 ml (19 November 1972).

Serum triglyceride 82 mg/100 ml (December 1972).

↓Serum thyroxine 4·9 μg/100 ml. Free thyroxine index 3·5 (normal: 3·5–8·0) (13 December 1972).

Lipoprotein electrophoresis; increase in β-lipoprotein, probably normal pattern for age (8 December 1972).

α-Fetoprotein; negative (12 December 1972). Australia antigen; negative.

Mitochondrial antibodies. Immunofluorescence; positive. Complement fixation titre >256.

ANF. Positive at 1/320. Antibodies to smooth muscle. Positive to 1/10 (11 December 1972).

ECG. Left axis deviation (8 December 1972).

Biopsy of liver (H4385/71). Portal system invasion by lymphocytes and plasma cells. Granulomata with macrophages forming giant cells. Normal architecture. Histology compatible with possible biliary cirrhosis.

Biopsy of lesion of right knee (H651/70). Cavernous haemangioma.

Electron microscopy. Normal endothelial cells and pericytes. No laminated inclusions so characteristic of Fabry's disease. Dermal capillaries and arterioles, and an isolated sweat duct, also show no inclusions (normal: glycosphingolipid of Fabry's disease appears as vacuoles in endothelial cells, pericytes and muscle cells).

(II) Dyskeratosis. For diagnosis.

DR R.P.WARIN

Bristol Children's Hospital, Bristol

R.W., female, aged 4 years.

History. Noted to have a thick, rough skin at birth. Napkin eruption at 3 weeks, with patches of raised, red, well demarcated skin showing features of napkin psoriasis. This was recurrent for 6 months.

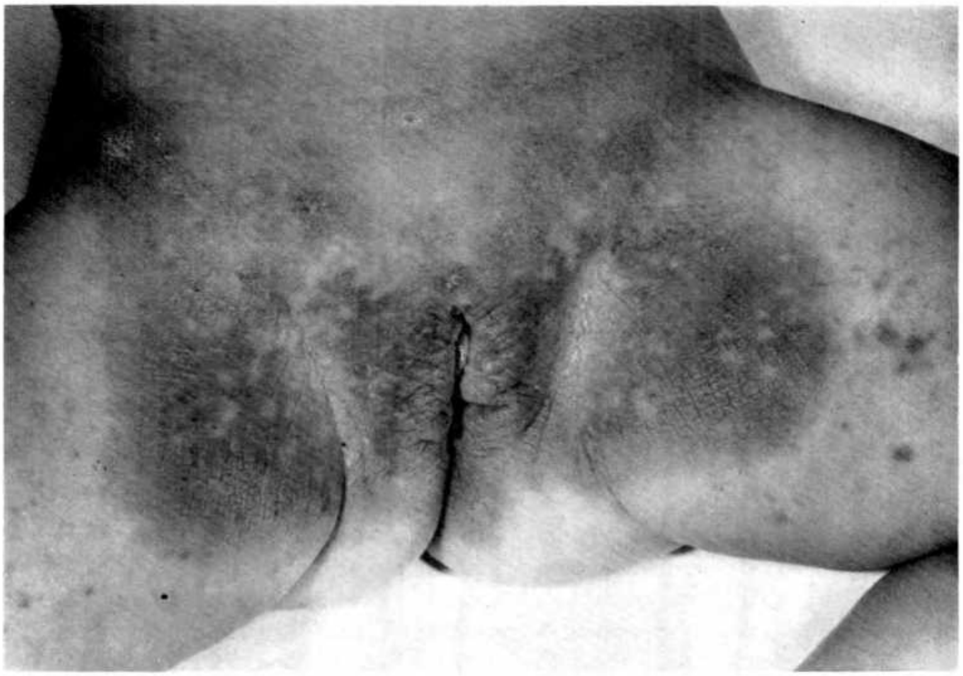

FIGURE 1. Diffuse hyperkeratosis in the groins.

Recurrent infections of the skin in the napkin area, around the mouth angles, eyelids, and nail folds.

In the last 2 years, a diffuse hyperkeratosis has developed in the axillae, on the front of the neck, round the umbilicus, in the groins and intergluteal cleft (Fig. 1), round the scalp margins, behind the ears, and over the centre of the back. Transient hyperkeratotic papules have developed, chiefly over the thighs.

Family history. No siblings. No family history of skin disease.

General health. Severe deafness, diminished vision, constipation, and frequent urinary infections.

Examination. Velvety, diffuse hyperkeratosis in areas mentioned. Hyperkeratotic papules over legs. Infected areas round mouth, buttocks, eyelids, and other sites. Left divergent strabismus.

Investigations. Bacteriology. Various organisms have been isolated from the skin, including Staphylococci, Streptococci, Pyocyaneus, and Candida species. *Histology.* Abnormal epidermis, which is thickened and thrown into crests, and troughs filled with excessive keratin showing a basket-weave pattern. Some hair follicles show dyskeratosis and keratin plugging, and there is a proliferation of the sweat duct units filled with PAS positive material, suggesting a naevoid abnormality. *Dermatoglyphics.* Complete absence of epidermal ridges on the fingers, palms, soles and toes. *Immunology.* Normal investigations, except some possible impairment of cell mediated immunity.

Comment. Has now developed some features of juvenile acanthosis nigricans, but there coexist a possible immunological deficiency state, absent dermatoglyphics, and the transient hyperkeratotic papules.

(12) Ectrodactyly, ectodermal dysplasia and cleft lip and palate (EEC syndrome)

MR J.N.SWALLOW, PROFESSOR O.P.GRAY AND DR P.S.HARPER

Dental Hospital, Department of Paediatrics and Department of Medicine, University Hospital of Wales, Cardiff

A.T., female, date of birth, 13 December 1969.

History. This child was the result of a normal full-term pregnancy and delivery, birth weight 6 lb 2 oz, with no history of maternal drug ingestion or infection during pregnancy. The parents were healthy and unrelated, the father being 25 and the mother 24 years old at the time of the child's birth; there were no previous pregnancies. Apart from a distant relative (first cousin of the maternal grandfather) with cleft lip, no other family members had been affected with any similar disorder.

At birth abnormalities of the hands and feet were noted; left cleft lip and palate were present, which were repaired aged 6 months and 13 months respectively. Scalp hair was sparse at birth and has remained both scanty and much fairer than that of the parents. The skin has been generally dry, with recurrent eczematous patches. There have been recurrent upper respiratory infections with persistent rhinitis, together with epiphora and photophobia. A single episode of urinary tract infection at the age of 2 years responded to antibiotics. Motor and mental development have been normal.

Examination. The limb defects are shown in Figs. 1 and 2. The abnormalities result from an asymmetrical combination of fusion, hypoplasia and absence of digits. The nails were pitted and dysplastic even on the normal digits. Scalp hair was sparse and fair but showed no tendency to breakage; eyebrows and eyelashes were scanty. The skin was fair and generally dry, but without obvious hyperkeratosis. Heart, lungs and abdomen were normal, and no skeletal abnormality was found outside the hands and feet. Height at 36 months was 86 cm (the 3rd percentile for this age). Ophthalmic examination under general anaesthetic (Mr M.V.Graham) showed bilateral stromal opacities in the cornea and a blocked right naso-lacrimal duct. Preliminary audiometric testing suggested a moderate, low frequency conductive deafness. General development quotient was average for her age (DQ = 90).

Dental examination. She had a left cleft lip and palate and a Skeletal 1 Incisal 1 jaw relationship. Upper left primary canine was in linguo-occlusion and in the palate. There was an intact primary dentition with fusion of $\overline{BA/AB}$. Primary molars were hypoplastic over their whole surface and pitted with sharp cusps. The incisal edge of the lower fused teeth was notched.

X-rays revealed apparently small first permanent molars, undeveloped secondary premolars, undeveloped permanent lower canines and central incisors, with very small lateral incisors. Upper incisors picture was confused because of the cleft.

Investigations. The urine showed no albumin or glucose, but 10–40 white cells per high power field and > 100,000 organisms per ml were present on several occasions; *Escherichia coli* was persistently grown on culture. Chest X-ray showed patchy consolidation of the right upper and lower zones. IVP showed a small left kidney; the right kidney was large and the bladder normal. Skeletal X-rays showed no abnormality outside the hands and feet. Other investigations included: Hb 11·2 g/100 ml, normal blood film, total serum proteins 7·5 g/100 ml with normal electrophoretic strip, blood urea 6·0 mmol/l, serum calcium 2·52 mmol/l.

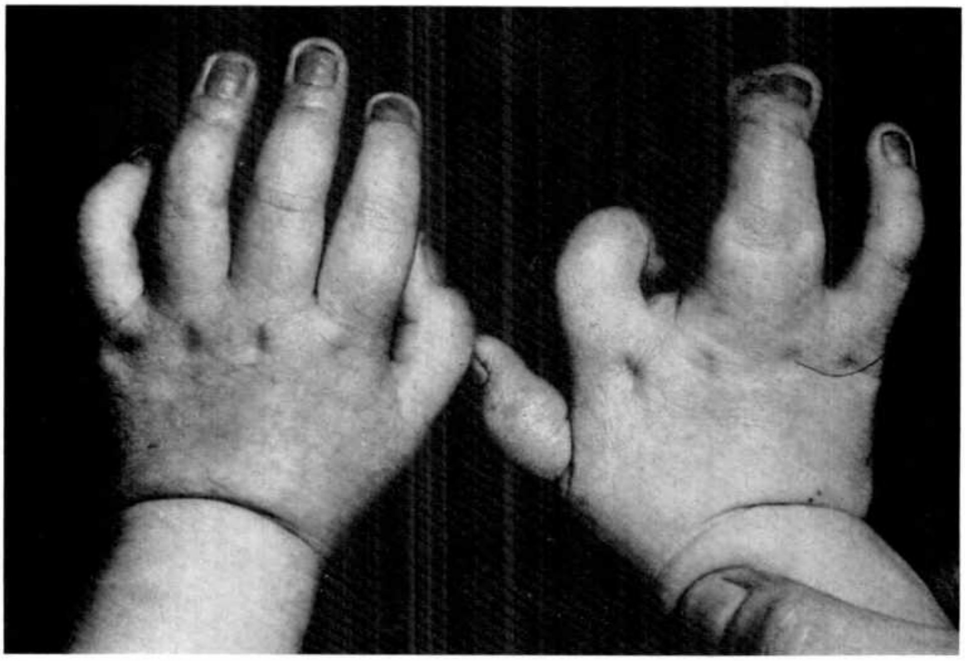

FIGURE 1. EEC syndrome to show hand deformity.

Discussion. It seems likely that this syndrome is a clinical and genetic entity, distinct from other types of ectodermal dysplasia. A family with ectrodactyly and cleft palate in two generations, described by Cockayne (1936), probably represented this condition, and the syndrome has been more fully delineated by the recent reports of Rudiger *et al.* (1970) and Bixler *et al.* (1971). In addition to the split hand and foot deformity, the cleft lip and palate, the dental dysplasia with hypodontia, and the sparse hair and dysplastic nails, other abnormalities have been noted. Recurrent keratitis, leading in some cases to corneal scarring, and accompanied by blockage of the naso-lacrimal duct, is a consistent feature; conductive deafness may occur (Robinson, Wildervanck & Chiang, 1973). Urinary tract abnormalities have also been noted, in particular hydronephrosis and hydroureter. All these features occurred in the present case, and their early detection is clearly of practical importance in the management of affected individuals. The finding of multifocal carcinoma *in situ* of cervix and vagina in an adult patient (Brill, Hsu & Hirschorn, 1972) raises the possibility that in this disorder, as in some other ectodermal dysplasias, the risk of epithelial neoplasia may be increased.

Although a high proportion of patients reported with this syndrome have been isolated cases, the recent reports of transmission through two generations (Brill *et al.*, 1972; Fried, 1972) strongly suggest simple Mendelian autosomal dominant inheritance; male to male transmission has not yet been reported, as X-linked dominant inheritance cannot be excluded.

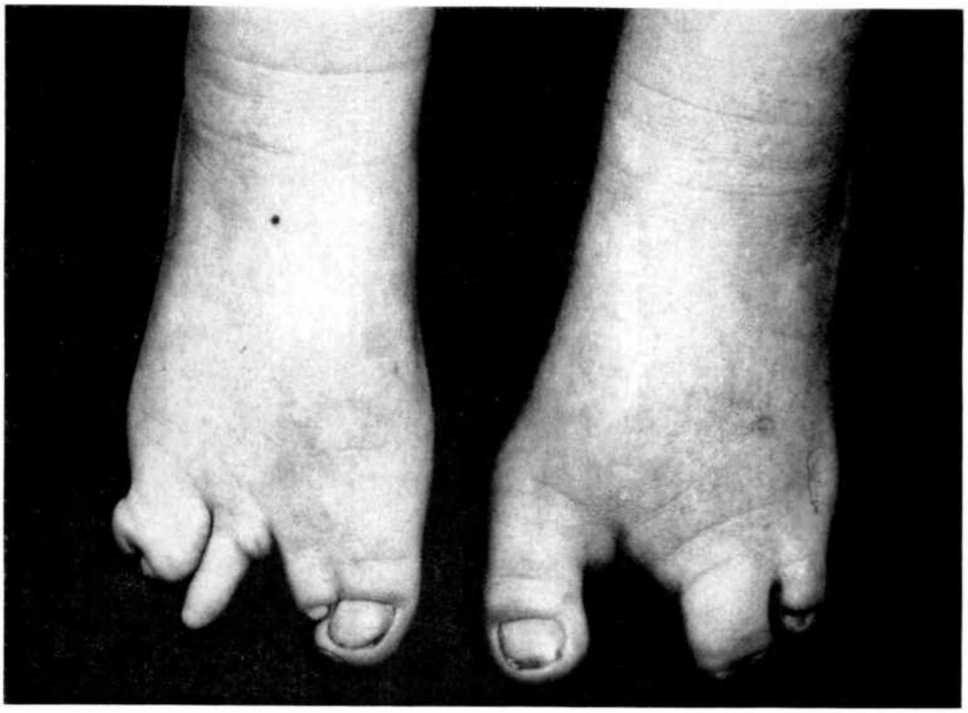

FIGURE 2. EEC syndrome, to show feet.

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(13) Congenital epidermolytic hyperkeratosis

DR RODERICK HOWELL

Cimla Hospital, Neath, Glamorganshire

J.H.L., male, aged 55 years.

History. His parents died when he was 7 years old, and the family history is not known. In infancy, he is said to have had blisters on his fingers and toes. In early childhood, there was gradual development of hyperkeratosis on the palms and soles. Blisters developed on the palms, soles, and on the neck and in the axillae in hot weather. From 12 to 37 years old he was in a chronic ward with severe hyperkeratosis of the palms and soles, which was removed surgically on several occasions without benefit. During adolescence numerous nodules developed on the trunk. He had no education or occupation.

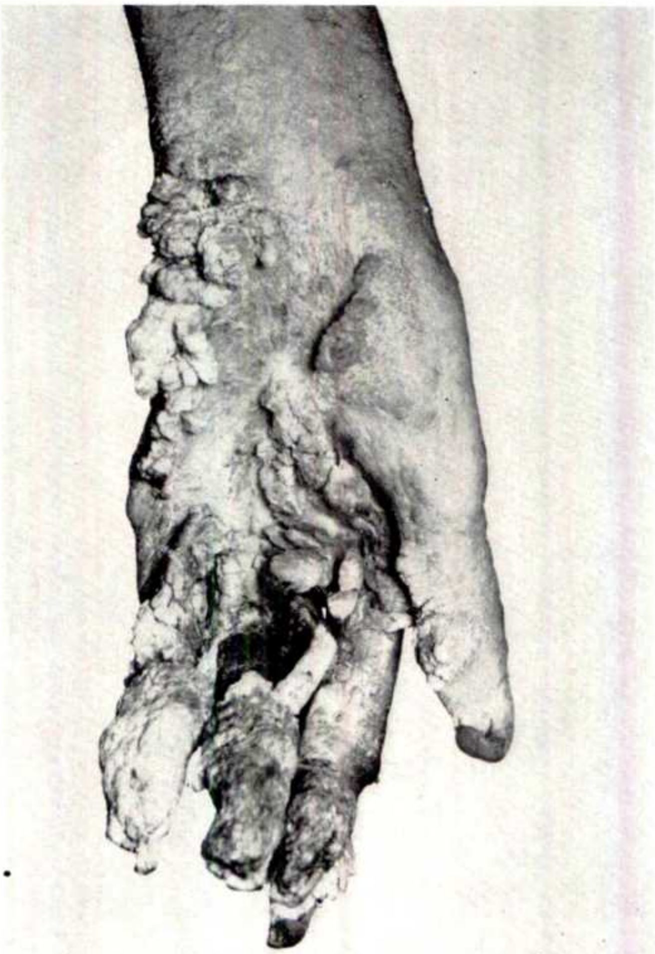

FIGURE 1. Gross hyperkeratosis of palms, fingers and nail folds.

In 1955 he was admitted to the Cardiff Royal Infirmary. At that time he had: (1) gross hyperkeratosis and occasional bullae on the palms, flexor aspect of the fingers, and nail folds (Fig. 1); (2) the

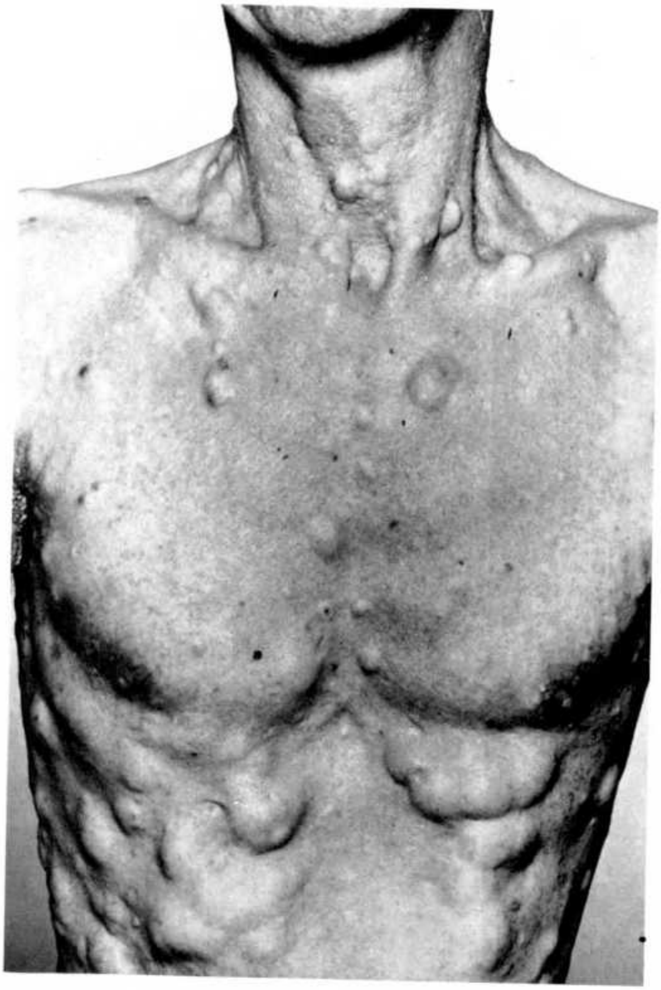

FIGURE 2. Multiple follicular hyperkeratosis, sebaceous cyst and lipomata.

feet were similarly affected; (3) on the trunk and limbs there were multiple follicular hyperkeratoses, many of them of the Kyrle type, numerous sebaceous cysts and lipomata (Fig. 2); (4) Hyperkeratotic plaques on the sides of his tongue (Fig. 3).

In November, 1955, he had a coronary thrombosis confirmed by ECG. Pain and deformity prevented any normal function of his hands and feet. He was cachectic, with disuse atrophy of muscles.

In March, 1956, excision and grafting of skin on the palms and soles was performed by Mr Emlyn Lewis. This enabled him to use his hands and feet effectively for the first time, and completely altered his life.

Examination. The appearance of his skin remains much the same as described in 1955, except for the improvement of the hands, and feet, resulting from plastic surgery.

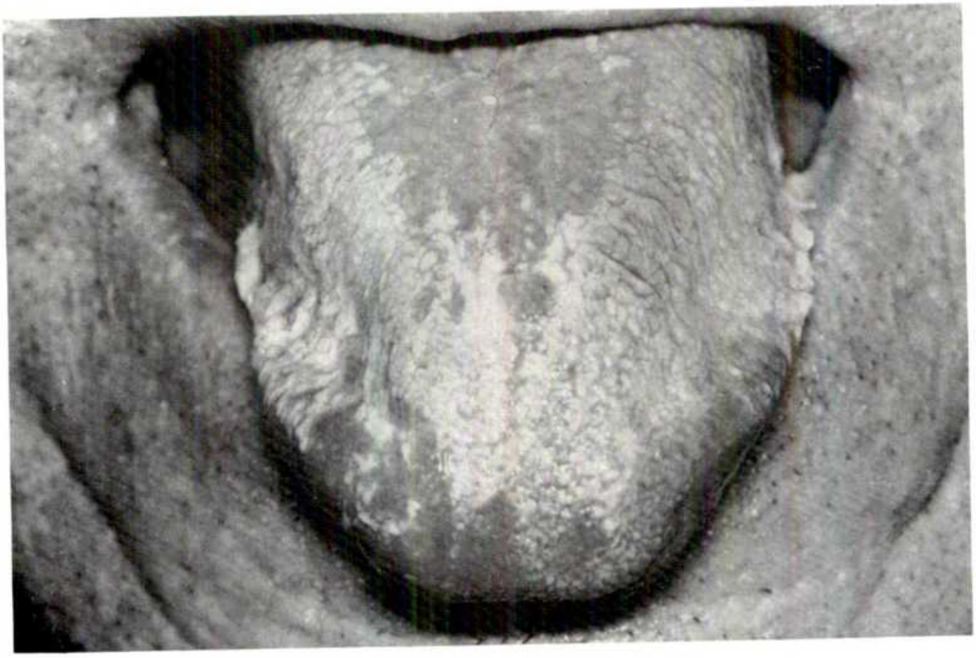

FIGURE 3. Hyperkeratotic lesions of the tongue.

Histopathological report (Dr Hubert Whiteley). The lesions are confined to the epidermis and show the disease in various stages of development. The initial lesion appears to be a localized epithelial hyperplasia similar to that seen in a virus wart, which develops vacuolation of the prickle and granular cells (Fig. 4) with the development of a sub-corneal bulla (Fig. 5) containing a few degenerate cells and capped by a zone of hyperkeratosis (Fig. 6).

Recent progress and treatment. For many years he has enjoyed an active life, living in a bungalow with television, telephone, and an invalid car, in which he travels great distances. A recent attack of cellulitis of the foot responded to antibiotics. He reports improvement of the hyperkeratoses with Calmurid cream.

Comment. He was shown at a British Association of Dermatology Meeting at Cardiff in 1958 as a case of congenital polykeratosis (Touraine). The histology indicates that a diagnosis of congenital epidermolytic hyperkeratosis is more appropriate. The plastic surgery which was done in 1956 to the palms and soles has allowed him to lead an active and socially integrated life.

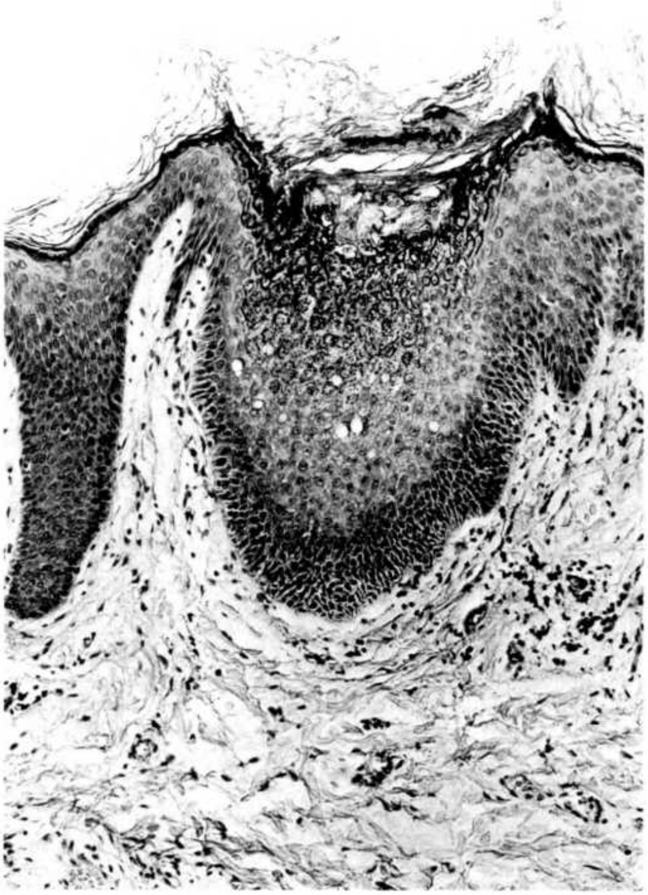

FIGURE 4. Epithelial hyperplasia with vacuolation of prickle and granular cells (× 295).

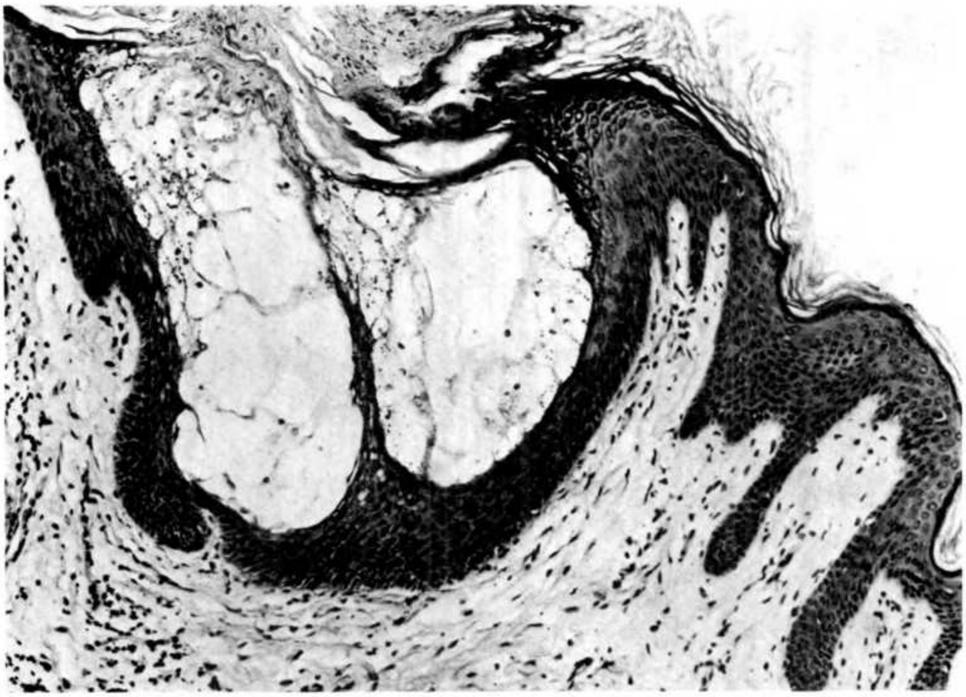

FIGURE 5. Development of a sub-corneal bulla (× 281).

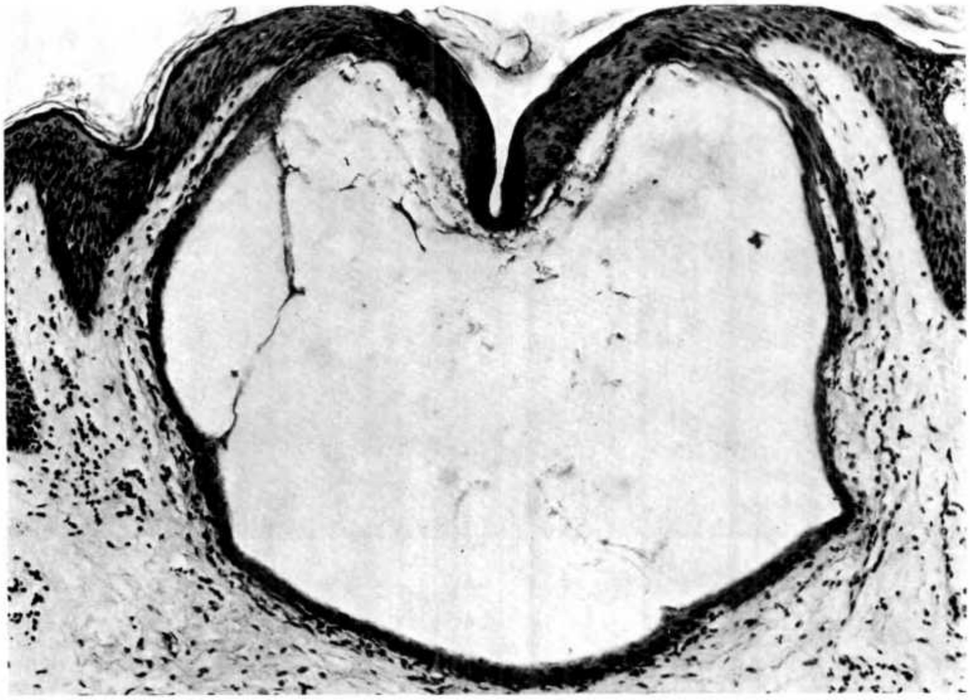

FIGURE 6. A fully developed sub-corneal bulla containing degenerate cells capped by a zone of hyperkeratosis (× 281).

(14) Ichthyosiform erythroderma variant

DR RODERICK HOWELL

Bridgend General Hospital, Bridgend

E.J.M., female, aged 16 months.

History. Born 27 February 1972 3 weeks premature. Birth weight 5 lb 11 oz. First child. No consanguinity of parents. No history of illness or drugs during pregnancy.

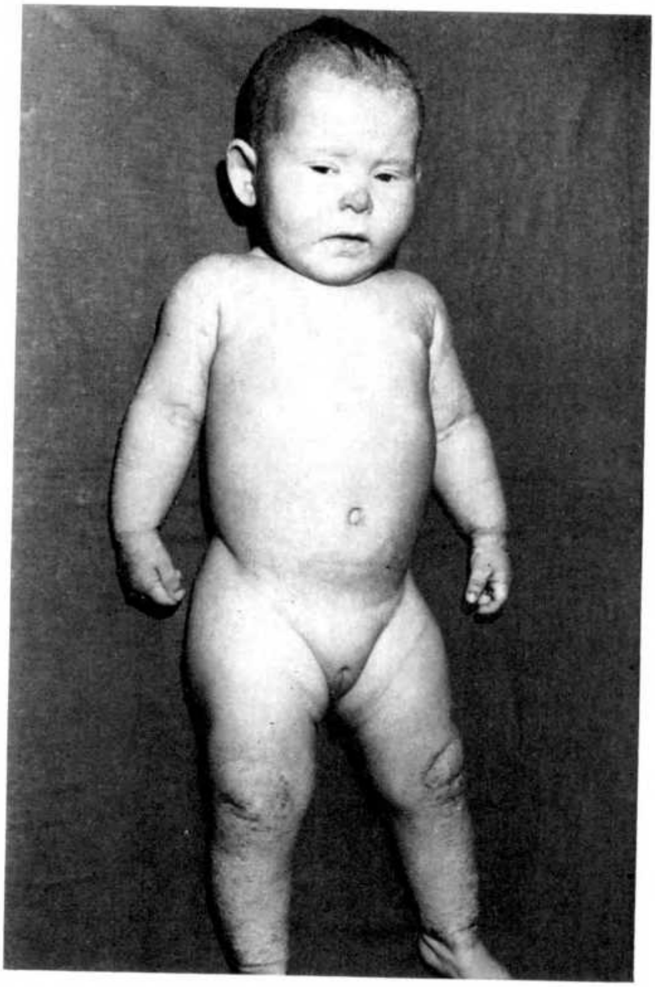

FIGURE 1. Widespread ichthyosis—some unaffected skin on the trunk.

At birth, no abnormality of skin noticed. At 10 days, mild peeling of skin. At 3 weeks, had thickening of the skin on the face, elbows and knees. This has become more widespread but is now stationary. No blisters seen at any time.

Her general health and development are not affected.

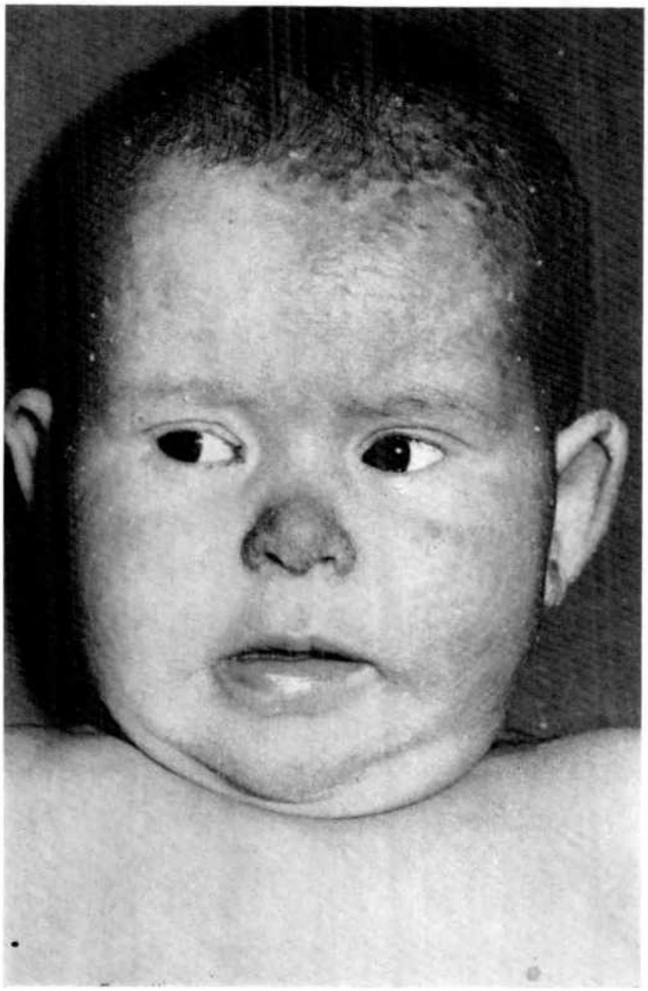

FIGURE 2. Diffuse ichthyosis on face. Most marked on the tip of the nose.

Examination. Widespread areas of ichthyosis on scalp, face, limbs and trunk (Figs. 1–3). Large areas of normal skin on trunk. Hair fine. Nails normal. Flexures of limbs affected. Palms and soles normal.

Treatment. Emollient creams only.

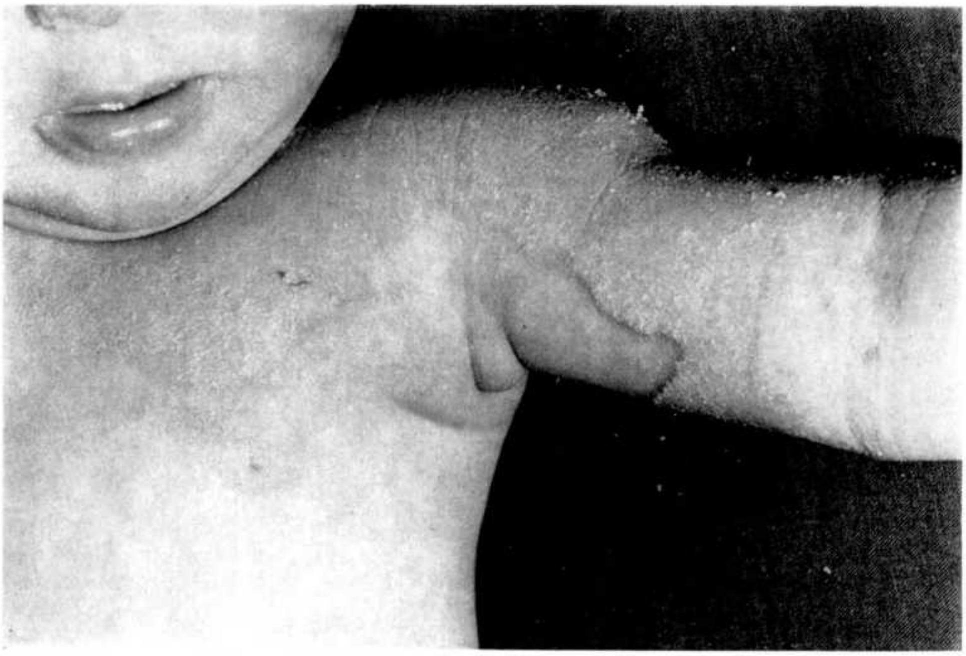

FIGURE 3. Note the sharply demarcated ichthyosis

Comment. Lack of movement of the lesions would appear to exclude erythrokeratoderma variabilis. A variant of non-bullous ichthyosiform erythroderma is a suggested diagnosis. Two other families with a similar pattern of ichthyosis, in which siblings are affected, have been observed (Wells, 1972).

REFERENCE

WELLS, R.S. (1972) Personal communication.

(15) Congenital ichthyosiform erythroderma

DR A. NAVARATNAM AND DR G. A. HODGSON

Department of Dermatology, University Hospital of Wales, Cardiff

J.S., male, aged 73 years.

History. Thickening of skin was noticed shortly after birth and has persisted. He has never worked and has never learned to read or write, because he could not attend school. He was first seen in our department in 1965, when he came to the skin clinic for 'a spot of ointment'. The skin has remained unchanged except for an increase in the warty excrescences, which are most marked on the back. The teeth and nails developed normally, but he lost his teeth from caries. He never had scalp hair. His eyesight was normal. There was no previous history of epilepsy. Ectropion had been present for as long as he could recollect.

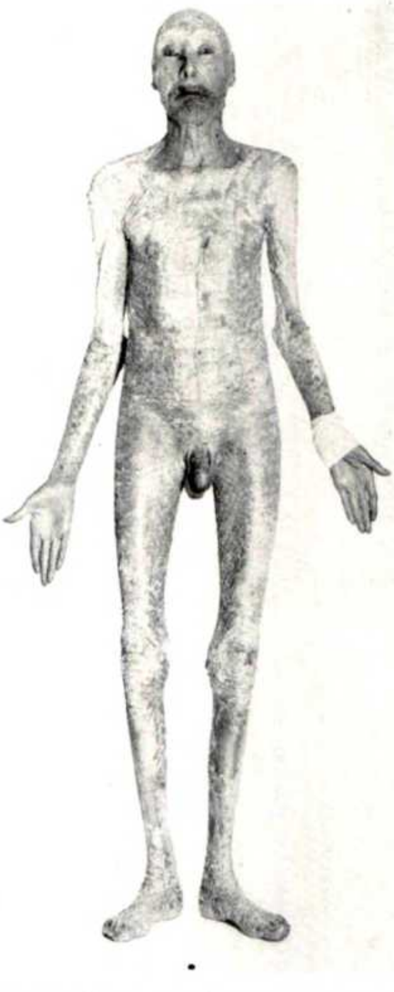

FIGURE 1. Gross hyperkeratosis of the skin.

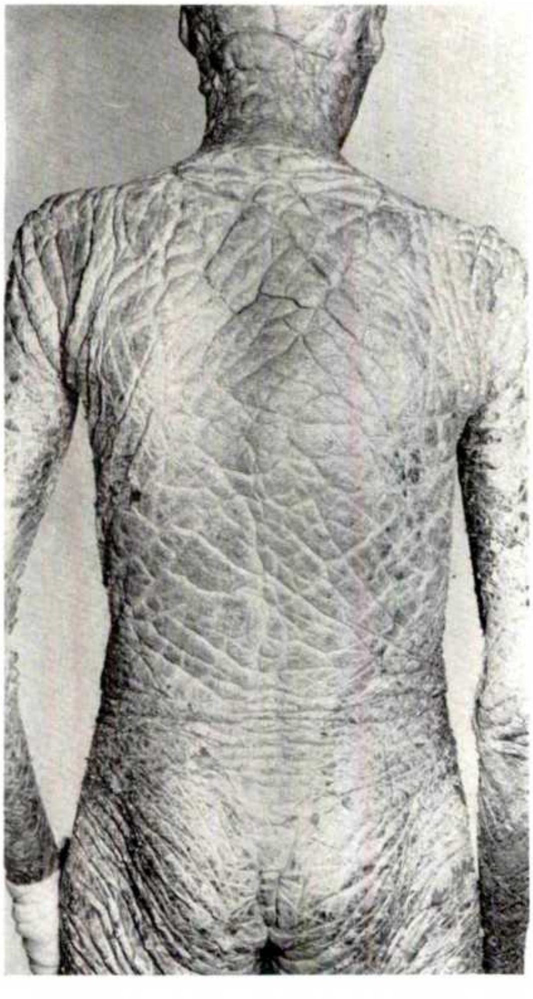

FIGURE 2. Gross hyperkeratosis of the skin.

Family history. The patient is the second in a family of eleven siblings. Eight died before the age of 14 years. One sister died in childbirth, aged 40 years, and one sister is alive and well.

Examination. There is gross hyperkeratosis over all areas of the skin (Figs. 1–3). The palmar skin is dry. The feet show some hyperkeratosis. The pinnae are closely approximated to the sides of the head.

Investigations (1973). Hb 13·3 g/100 ml. WBC 7000/mm^3. Normal differential. ESR 24 mm/1 h. Liver function tests normal. Plasma proteins normal. Electrolytes normal. Chest X-ray showed changes in keeping with bronchitis, and emphysema. Skin biopsy; gross hyperkeratosis with epithelial hyperplasia. (Fig. 4).

Course and Treatment. There was symptomatic improvement with topical treatment. The best results were obtained from salicylic acid under occlusion, after which skin remained smooth for about 9 months before hyperkeratosis once more built up. Hypnosis produced temporary 'marginal' improvement. In 1967, he had a spell of dyspnoea and was diagnosed as bronchitis with emphysema.

Comment. Though he is unable to read or write, the patient is mentally alert with good eyesight, and has no neurological defects. He is happy, cheerful, and lives, with his nephew, on national assistance. Ichthyotic diseases comprise a broad group of related disorders varying much in severity and distribution and are often associated with other anomalies. Although progressive in childhood, many cases stabilize or improve in later years.

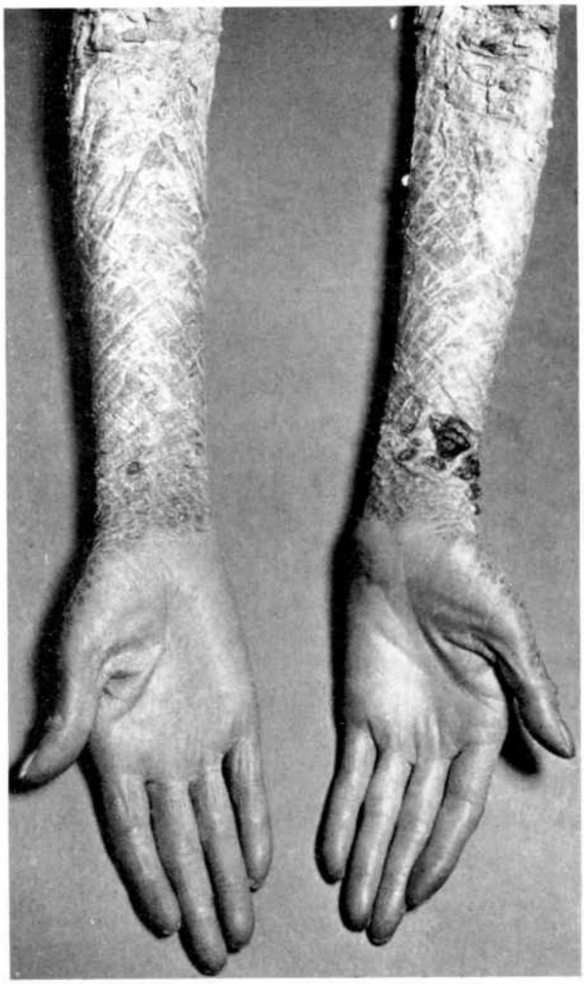

FIGURE 3. Gross hyperkeratosis of the skin.

This type of icthyosis would have been placed by Riecke (1900) in Group 3 of ichthyosis congenita. On clinical and genetic findings it could be called congenital ichthyosiform erythroderma non-bullous type (Reed, 1970).

In some ichthyosiform eruptions, marking studies reveal rapid epidermal cell turn-over, even more than in psoriasis (Marks, 1973). Such studies have not been carried out on our patient, but it is interesting to note that it is some months before the ichthyosis recurs after its removal in the occlusion, suggesting a slow epidermal turn-over.

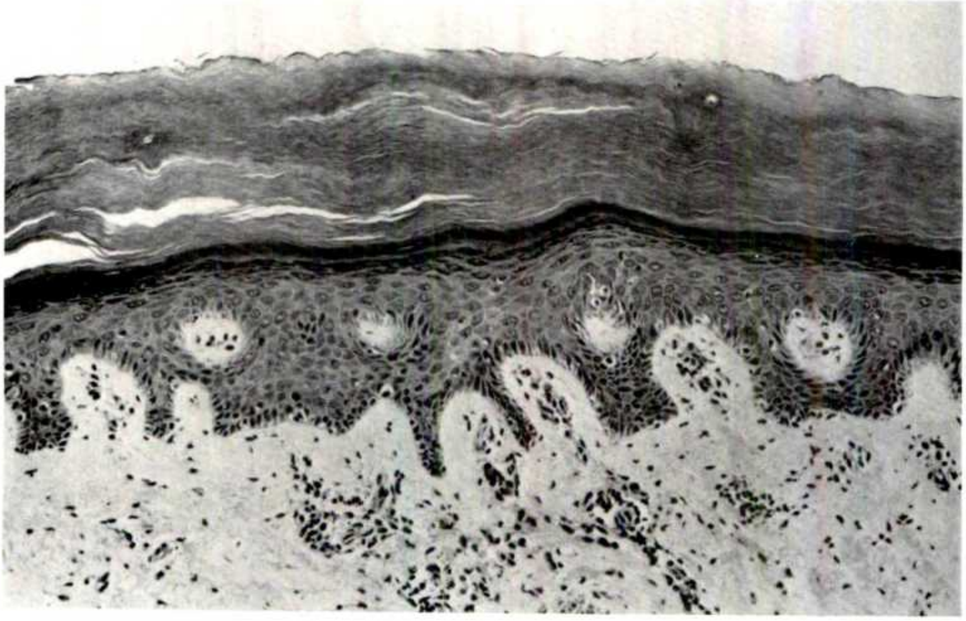

FIGURE 4. Skin biopsy—marked hyperkeratosis and epithelial hyperplasia (× 224).

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(16) Recessive dystrophic epidermolysis bullosa?

DR A. CATTELL AND DR E. WADDINGTON

Department of Dermatology, University Hospital of Wales, Cardiff

Mrs D.G., aged 59 years. Housewife.

History. At the age of 18 the patient developed on the light exposed areas a papular eruption which recurred in the summer months. In 1968, there was a sudden onset of a widespread bullous eruption on the limbs, trunk and face, and she had severe ulceration in the mouth. In 1971, she was first seen in Cardiff. She had developed widespread skin erosions, she had dysphagia, had lost weight and had recently lost finger and toe nails. Blisters appeared at sites of minor trauma.

Family history. Mother died aged 35. Father killed in the 1914–18 War. One brother, aged 54, alive and well. One brother died aged 54. Her three daughters are alive and well. No history of any skin disease. No consanguinity.

Examination. She was cachectic. Weight 32 kg. *Skin.* There were multiple denuded areas, 1–5 cm in diameter, and both clear and haemorrhagic flaccid bullae on the face, limbs, abdomen, back and buttocks (Fig. 1). Nikolsky's sign was positive. There was widespread atrophic scarring with large numbers of milia. The finger tips were sclerotic, and finger and toe nails dystrophic (Figs. 2 and 3). There was microstomia. The tongue was smooth and ulcerated and there were numerous erosions on the buccal mucosae with obliteration of the gingivo-labial sulci. Edentulous. The scalp and body hair was sparse. No conjunctival lesions. Other systems normal.

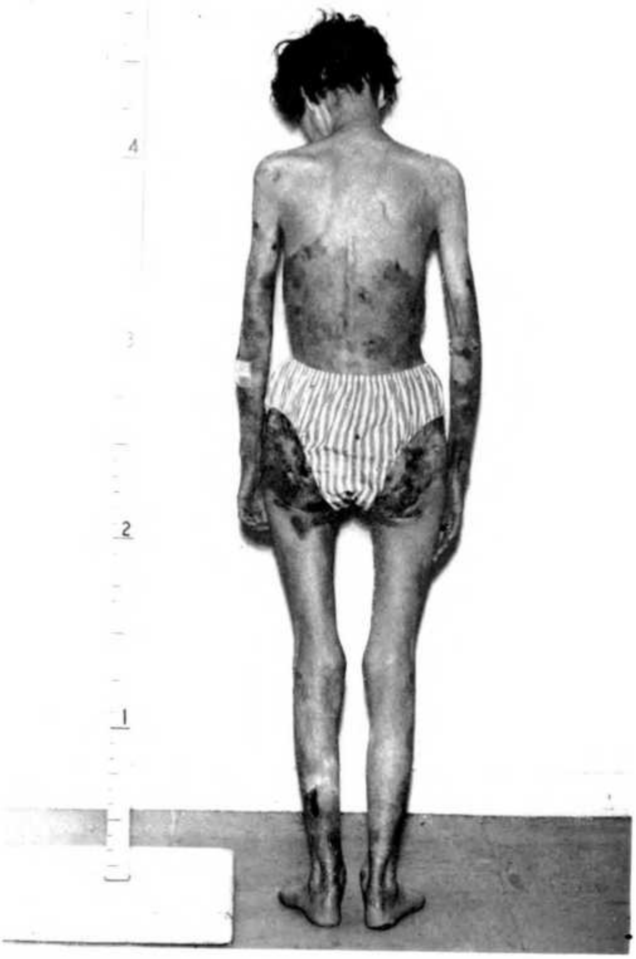

FIGURE 1. Widespread erosions on arms, trunk and lower legs.

Investigations. Hb 9·9 g/100 ml. WBC 19,700/mm^3. ESR 51 mm/1 h. Blood urea, serum electrolytes,

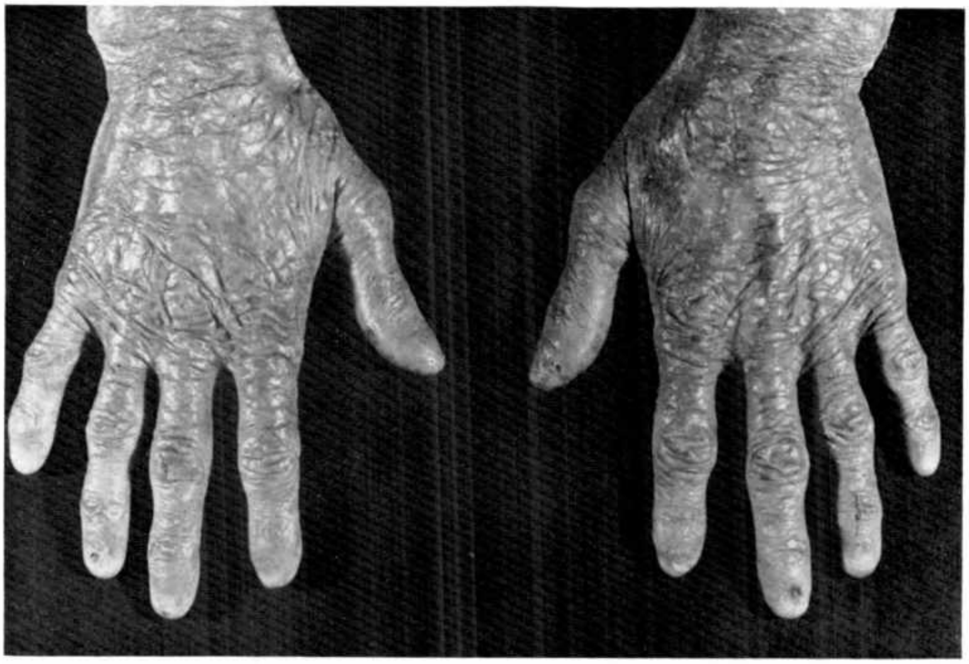

FIGURE 2. Hands. Dystrophic nails, severe atrophic scarring and multiple milia.

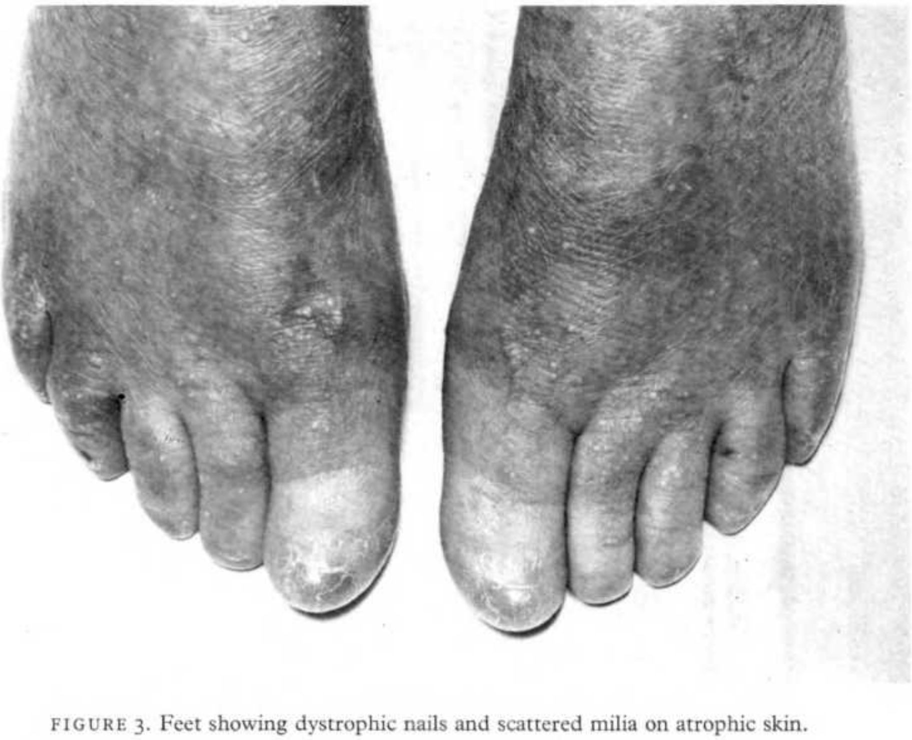

FIGURE 3. Feet showing dystrophic nails and scattered milia on atrophic skin.

immunoglobulins, electrophoresis, liver function tests, faecal fats, and serum vitamin B_{12} all normal. Repeated examinations have excluded porphyria. EEG normal.

Skin biopsy. Sub-epidermal bulla with minimal inflammation in the dermis. There is a line of polymorphs on the dermal side of the bulla. Direct and indirect immunofluorescence; negative. Electron microscopy; report awaited. *X-ray chest.* No abnormality. *Barium swallow.* There is a long smooth stricture in the upper third of the oesophagus (Fig. 4). The appearance suggests a benign fibrous stricture. *Oesophagoscopy* confirmed a fibrous stricture at 24 cm.

Treatment and progress. Prednisone therapy, up to 80 mg daily, gradually reducing to 10 mg daily produced a marked improvement. The erosions and bullae disappeared leaving atrophic scars and multiple milia. During the last 2 years relapses have occurred when the dose of prednisone has been reduced below 10 mg daily. She has recently had more severe dysphagia and indigestion. Barium meal; 'oesophageal stricture present, but smaller. There are coarse mucosal folds on the greater curvature of the stomach.' Investigation by fibroscope is intended (10 May, 1973).

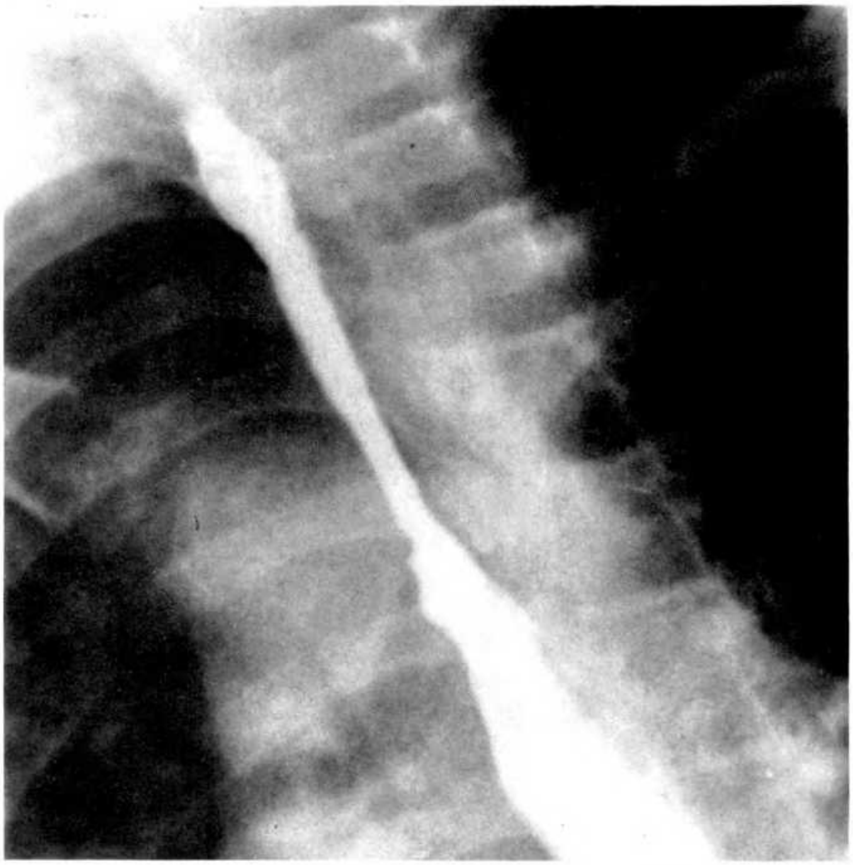

FIGURE 4. Barium swallow showing the long smooth stricture in the oesophagus.

Comment. This patient is exceptional because of the onset of epidermolysis in the fifth decade. Corticosteroid therapy (Moynahan, 1968) undoubtedly relieved both skin and mucosal lesions, but the more recent severe dysphagia will require a higher dosage of prednisone. The radiological findings in the stomach may indicate a carcinoma, and this will be investigated.

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MOYNAHAN, E.J. (1968) Corticosteroids in the treatment of severe dystrophic epidermolysis bullosa. *Proceedings of the XIII International Congress of Dermatology*, vol. 1, p. 208.

(17) Scleredema and diabetes mellitus

DR A. CATTELL AND DR E. WADDINGTON

Department of Dermatology, University Hospital of Wales, Cardiff

G.P., male, aged 43 years.

History. Since 1971 he has had difficulty in extending his neck and in raising his arms above his head. His neck has increased in diamater by 2·5 cm during the last 2 years.

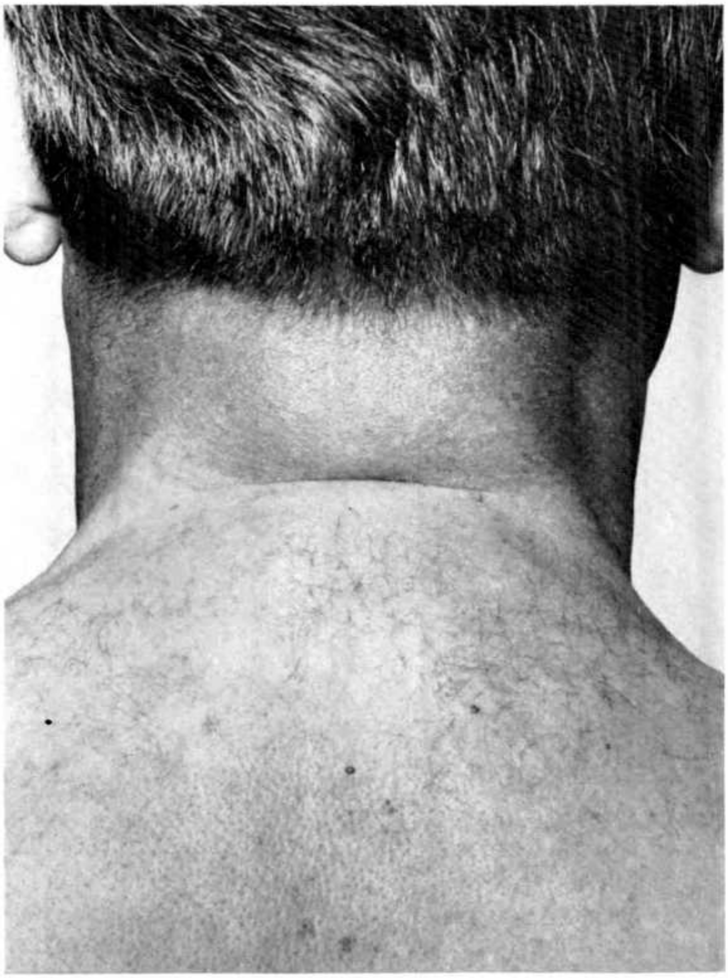

FIGURE 1. Note prominent crease caused by gross induration of the skin on the back of the neck.

He has been an insulin-dependent diabetic for 28 years. Recently the diabetes has been difficult to control. He is at present receiving insulin novolente, 52 units daily.

Past history. Since 1957: recurrent pyelonephritis. 1967: hypertension diagnosed—is receiving clonidine 0·2 mg three times daily. Progressive diabetic retinopathy.

Family history. His father was diabetic, and his paternal uncle is diabetic. His mother had coronary artery disease.

Examination. The patient was obese. The skin over the back of his neck, upper back and upper arms was congested and markedly indurated (Figs. 1 and 2). The edges of the indurated areas were difficult to determine. The skin elsewhere was normal, as was his tongue. Cardiovascular system: BP 220/120 mmHg. Fundi—exudates, haemorrhages and microaneurysms. There was evidence of diabetic and hypertensive retinopathy. In other systems, there abnormality was detected.

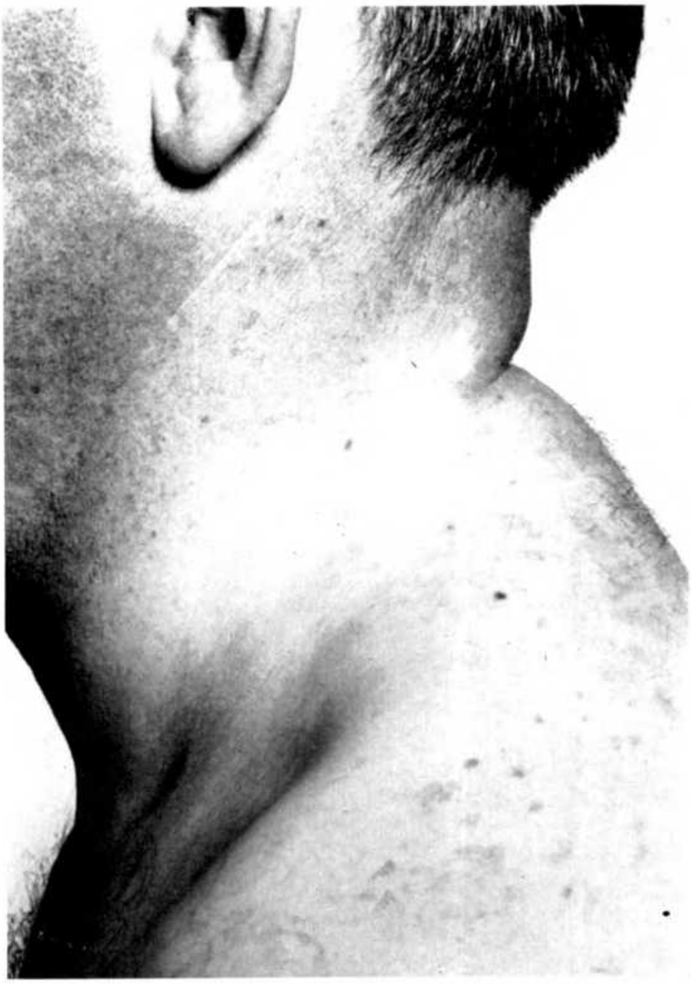

FIGURE 2. Induration on the shoulders simulating a 'buffalo hump'.

Investigations. Random blood sugar 12·5 mmol/l. Biochemical profile normal. EMG normal.

ECG; left axis deviation, left ventricular hypertrophy and partial right bundle branch block. Creatinine clearance 110 ml/min. MSU and sputum normal. X-ray chest and skull—no abnormality.

Biopsy of skin. Normal epidermis. The collagen bundles do not appear to be oedematous. There is possibly an increased amount of collagen compared with the matched controls. Alcian blue at pH 4 and 2·5 are not helpful, nor is the PAS stain. Laking of mucopolysaccharide is not observed.

Comment. The association of scleredema with diabetes mellitus is well documented (Fleischmajer, Faludi & Krol, 1970; Cohn, Wheeler & Briggaman, 1970). There appear to be three clinical groups of scleredema: (1) the classical group, which has an acute onset, follows an acute infection and may resolve in a few months; (2) the most common group, which has an insidious onset without preceding illness and persists for many years; (3) a group restricted to adults who have long-standing diabetes, associated angiopathies and cardiac and renal disease.

Our patient is typical of the last group. The failure to demonstrate an excess of mucopolysaccharide in some cases of scleredema has been noted by several authors; Cohn *et al.* (1970), Curtis & Shulak (1965), Fleischmajer & Lara (1965).

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(18) Systemic sclerosis and vitamin D intoxication

DR A. CATTELL, DR H.F. MOLLOY AND DR E. WADDINGTON

Department of Dermatology, University Hospital of Wales, Cardiff

E.D., female, aged 57 years. Housewife.

History. 1966, onset of Raynaud's phenomenon in both hands; 1967, ulceration of finger tips; 1968, first admission to hospital.

Examination. Sclerodermatous changes of hands, with ulceration of finger tips. Otherwise clinically well.

Investigations. Hb 10·9 g/100 ml. ESR 24 mm/h. Serum electrophoresis—raised gamma-globulin fraction. Chest X-ray—normal. X-ray hands—calcinosis at tips of left and right fourth fingers. Barium meal—early sclerodermatous changes; follow through meal, normal.

The diagnosis of systemic sclerosis was made, and the patient discharged without treatment.

June, 1971. Deterioration in general health. The patient noticed hard swellings on the extensor surface of right forearm, and on the back of the right thigh.

July, 1971. Admitted for further investigation.

Examination. The patient looked ill; she had a mask-like face, beaked nose and puckered lips (Fig. 1). There were areas of hyperpigmentation on the face and hands. There were marked sclerodermatous changes of hands with scarring of finger pulps (Fig. 2). There was a discharging sinus at the base of the right thumb. Movements of the right wrist were greatly restricted. There was a large firm subcutaneous mass on the dorsum of the right forearm, and a similar mass in the muscles of the back of the thigh associated with limitation of movement of the right knee. There were multiple subcutaneous calcified nodules in the right antecubital fossa and in both popliteal fossae. There was a hard mass overlying and below the left mastoid process. There were marked hypercalcaemic keratopathy and scleral calcification in both eyes. Examination of other systems revealed no abnormality.

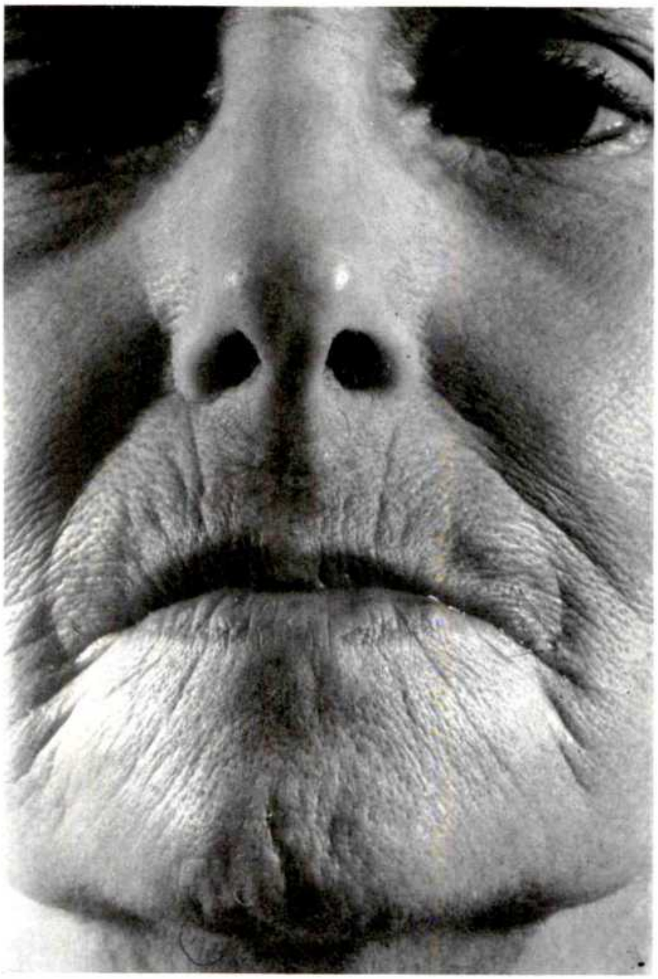


FIGURE 1. Typical puckering of the mouth.

Investigations. Hb 8·7 g/100 ml. ESR 89 mm/1 h. WBC 6000/mm^3. Blood urea 102 mg/100 ml. Creatinine clearance 9·8 ml/min. 24 h urinary calcium 359 mg—proteinuria. Serum calcium 12·6 mg/100 ml on many occasions. Serum phosphate 5 mg/100 ml. Serum alkaline phosphatase 38 KA units.

Chest X-ray—normal. X-ray hand—extensive soft tissue calcification (Fig. 3). X-ray right forearm—extensive muscular calcification (Fig. 4). X-ray renal areas—extensive calcification, reduction in kidney size. X-ray right thigh—extensive muscular calcification (Fig. 5). Barium meal—marked sclerodermatous changes in oesophagus.

Renal biopsy. Ischaemic type of sclerosis evident with matching severe hyalinization of arterioles, and severe intimal hyperplasia of medium-sized arteries, diagnostic of sclerodermatous involvement. The biopsy was difficult to cut because of widespread distribution of calcium.

In view of the persistently raised serum calcium levels, exploration of the parathyroid glands was undertaken. This revealed three normal parathyroid glands; histology of one removed—normal.

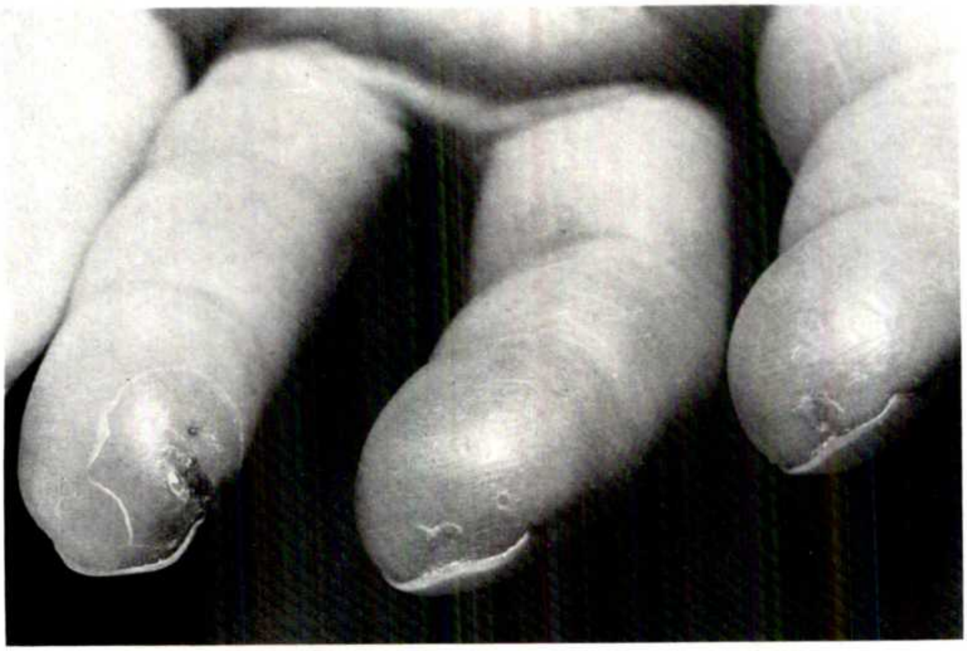


FIGURE 2. Acrosclerosis and ulceration of the finger tips.

Aspiration of mass in forearm—calcium phosphate.

Progress. After parathyroid exploration, there was a dramatic drop to normal in the serum calcium level. However, the serum calcium level soon began to climb to reach former levels by the fourth day. Two weeks post-operatively prednisone 30 mg daily was started. This brought considerable improvement, with: (1) return of calcium and phosphate levels to normal; (2) rise of creatinine clearance to 28 ml/min; (3) softening of hard swellings in muscle.

It was at this stage (October, 1971), on further close questioning that the patient's husband admitted that, although she had been a little lax about taking her calcium tablets, she had never missed her twice weekly injections of vitamin D (600,000 units), prescribed by her general practitioner for 'chilblains' since 1969. The patient has been maintained on a small dose of prednisone, and remains well. The subcutaneous calcification has diminished.

Discussion. The problems of this case, which we initially thought to be unique, were resolved. Tuffanelli & Winkelmann (1961), reviewing 727 cases of systemic sclerosis from the Mayo Clinic,

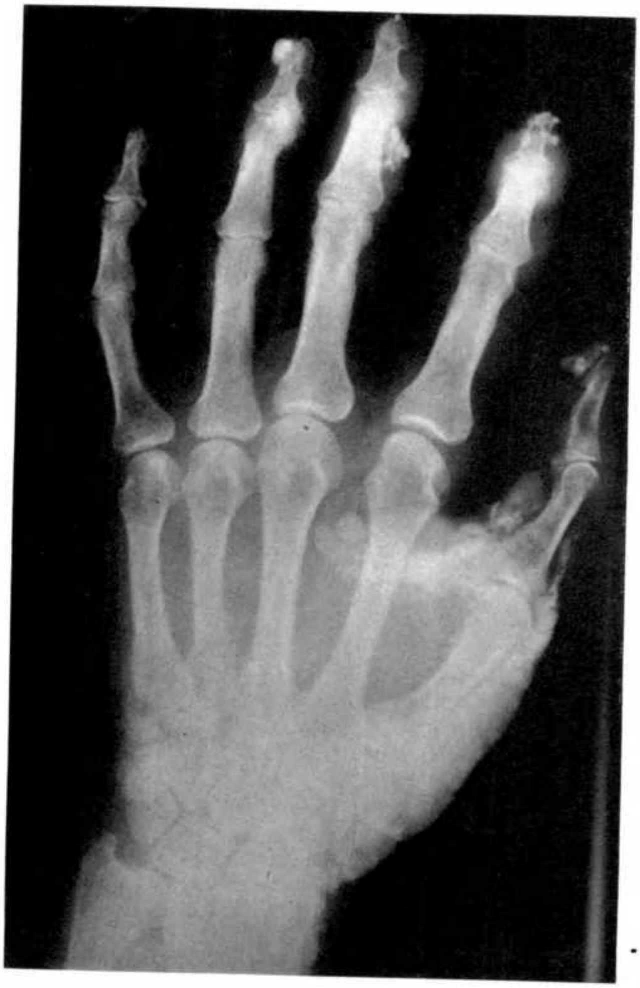
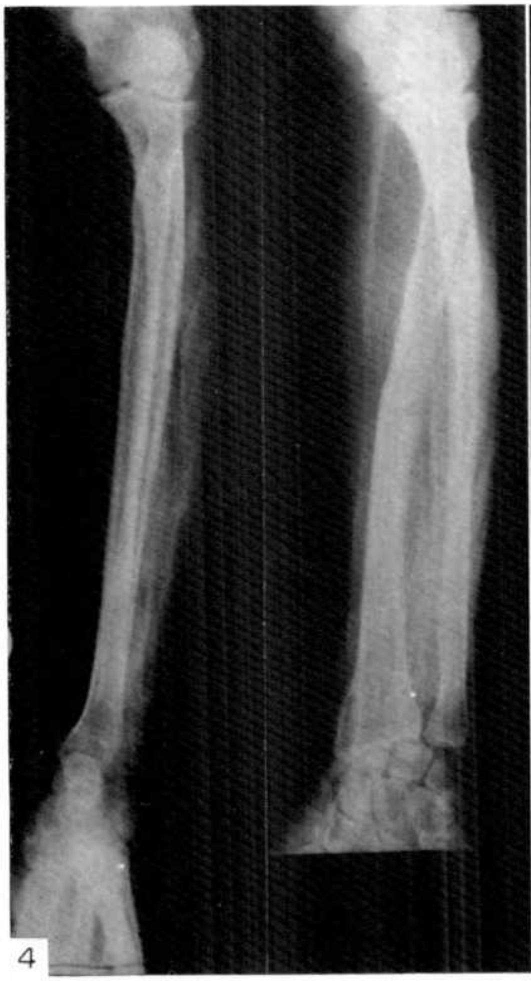


FIGURE 3. Extensive soft tissue calcification of the hand.

reported serum calcium estimations in 173 patients—all were normal except one woman, with an elevated serum calcium, who had hyperparathyroidism. Calcinosis is a late sequela of scleroderma, occurring, on average, 11 years after the onset of the disease, as noted in the Mayo Clinic survey. The serum calcium levels do not rise in association with the calcinosis. Treatment of systemic sclerosis is still debated, because of the risk of precipitating renal failure. According to Tuffanelli & Winkelmann (1961), their evidence suggests, but does not prove, that steroid therapy is dangerous in systemic sclerosis. This patient has obvious renal involvement with systemic sclerosis. However, far from precipitating renal failure, prednisone has improved renal function and she has remained normotensive.



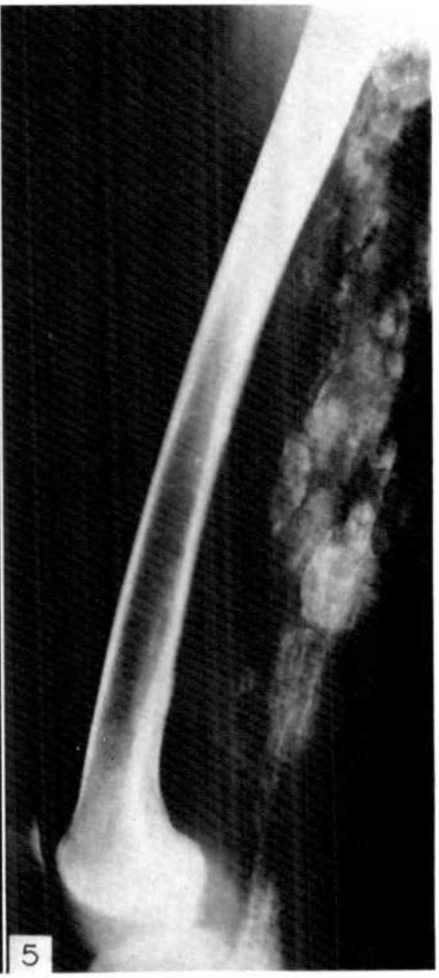


FIGURE 4. Extensive calcification of muscle on the right forearm.
FIGURE 5. Extensive calcification on the posterior aspect of the right thigh

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(19) Benign systemic mastocytosis

Dr A. Navaratnam and Dr G. A. Hodgson

Department of Dermatology, University Hospital of Wales, Cardiff

I.S., male, aged 56 years.

History. A Yugoslavian miner from Aberdare, the patient noticed on his back in 1971 a brown irritating rash, which spread to the trunk, face, limbs and chest.

Past medical history. Cervical osteoarthritis followed an accident in 1971. He suffered from bronchial asthma at 16 years. There was no previous history of jaundice and no relevant drug history. The patient takes very little alcohol.

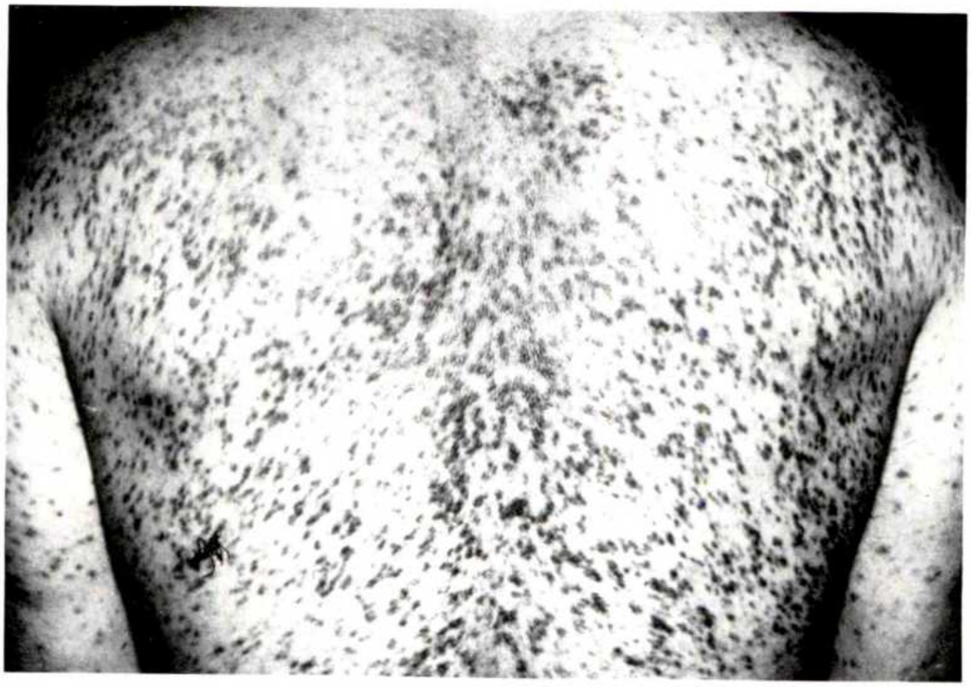

FIGURE 1. Skin showing brownish-grey macular eruption.

On examination. The patient is very sallow, but has no clinical jaundice. There is a brownish-grey, macular eruption mainly on the trunk, and to a less extent on the limbs (Fig. 1), urticating on friction. The liver is palpable 7·5 cm below the costal margin, with a well defined edge. The spleen is not palpable.

Investigations (17 May, 1972). Total proteins 87 g/l. Albumin 48 g/l. Bilirubin 45 mmol/l (normal range 0–17 mmol/l). Serum enzymes; SGPT 45 IU/l (5–28 IU/l normal range). SHBD 133 IU/l (0–360 IU/l normal range). Serum cholesterol 4·8 mmol/l (normal). Electrolytes normal. Serum calcium 2·6 mmol/l (normal). Alkaline phosphatase 30 IU/l (normal). Liver function test (22 May 1972). Total proteins 91 g/l. Bilirubin 34 mmol/l. Direct bilirubin 17·8 mmol/l. Indirect bilirubin 35·9 mmol/l. Immunoassay, IgG 2100 mg/100 ml (normal up to 1300 mg/100 ml), IgM 62 mg/100 ml, IgA 370 mg/100 ml. Direct Coombs test was negative. Coagulation studies were normal.

X-rays. Skeletal survey relatively normal apart from degenerative changes, suggestive of osteoarthritis of the cervical dorsal and lumbar spine. Chest X-ray normal.

Skin biopsy. Marked pigmentation of the basal layer, and focal aggregation of mast cells in the upper dermis (Fig. 2). Appearances are those of urticaria pigmentosa.

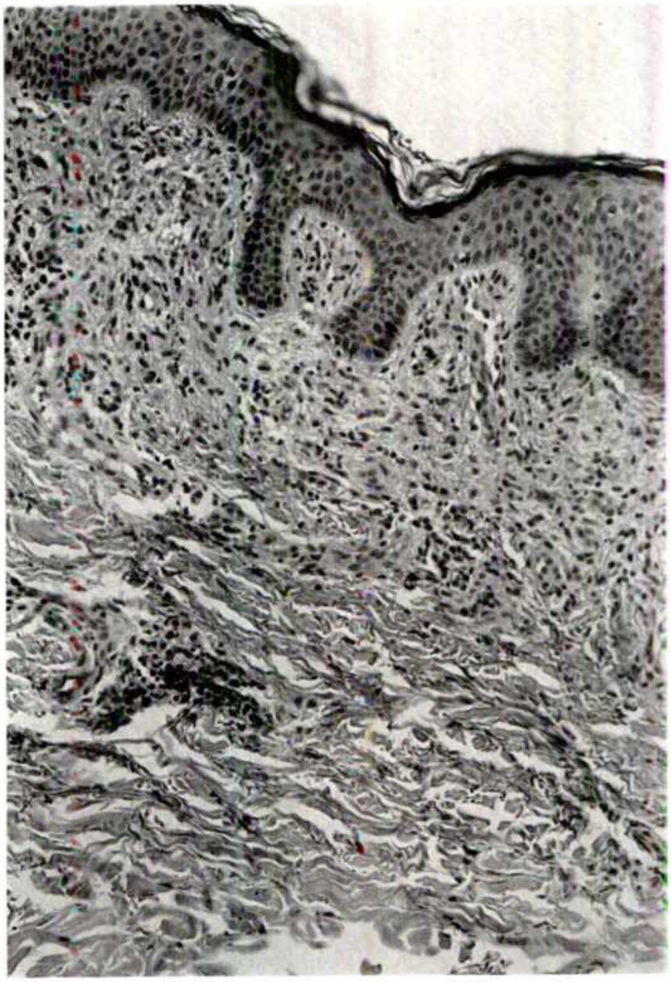

FIGURE 2. Skin biopsy, showing pigmentation of basal layer and focal aggregation of mast in the upper dermis.

Liver biopsy. Scanty portal tract, round cell infiltrate and occasional metachromatic granules. Scattered liver cells showed giant nuclei, indicative of hyperplasia (Fig. 3).

Course and treatment. There was no evidence of neurological disorder. Follow up studies showed that liver function tests had returned to normal. There was symptomatic improvement with antihistamines, as found by Moynahan (1950). Local Grenz ray on one arm was ineffective.

Comment. Urticaria pigmentosa was first described by Nettleship (1869). It is a rare disorder which accounted for one in every 2500 new patients at St Bartholomew's Hospital, London (Havard & Scott, 1959). Post-mortem studies by Ellis (1949) in a child 1 year old first established the systemic nature of the disease. Our patient fits into the category of benign systemic mastocytosis with skin and liver involvement but no evidence of mast cells in bone marrow. The actual number of cases with signs and symptoms is small and the incidence of systemic involvement has been estimated at 10% in a series of 145 such cases (Sutter, Beaulieu & Birtar, 1962). Most cases of systemic mastocytosis follow a benign course, but data collected by Sagher & Even-Paz (1967) indicated that leukaemia or a related condition affecting the reticulo-endothelial system developed in 24% of such patients.

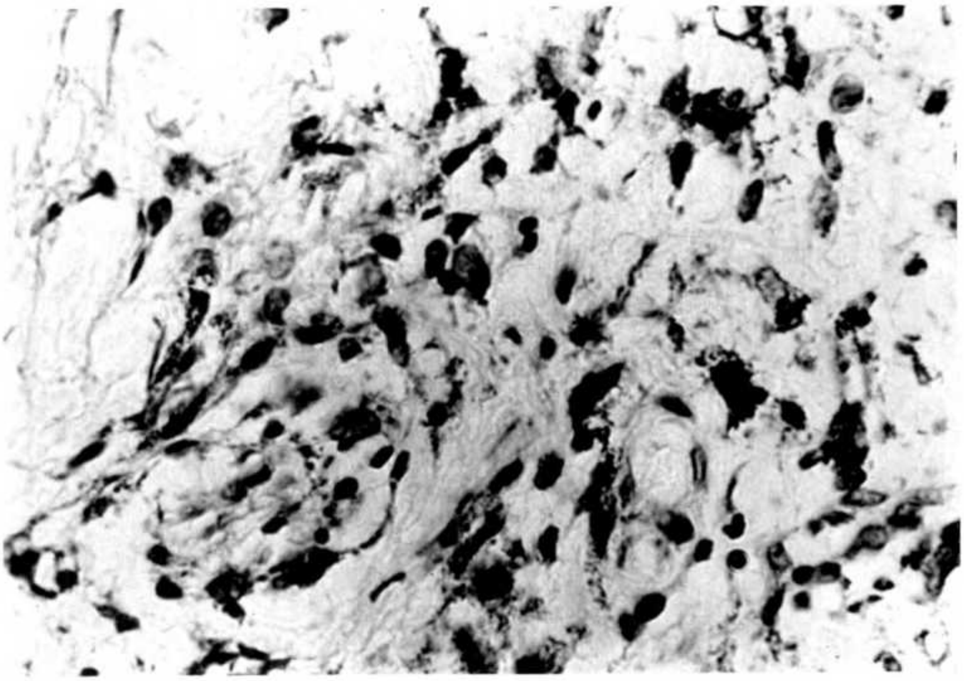

FIGURE 3. Liver biopsy showing slight periportal cellular infiltration.

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(20) Sarcoidosis and thrombocytopenic purpura

DR P.J.A.HOLT, DR C.A.J.WARDROP AND DR E.WADDINGTON

Departments of Dermatology and Haematology, University Hospital of Wales, Cardiff

V.A.J., female, aged 63 years. Housewife.

History. In July, 1970, the patient attended her local hospital with a rash on the nose and cheeks, which was diagnosed as rosacea and treated with oxytetracycline.

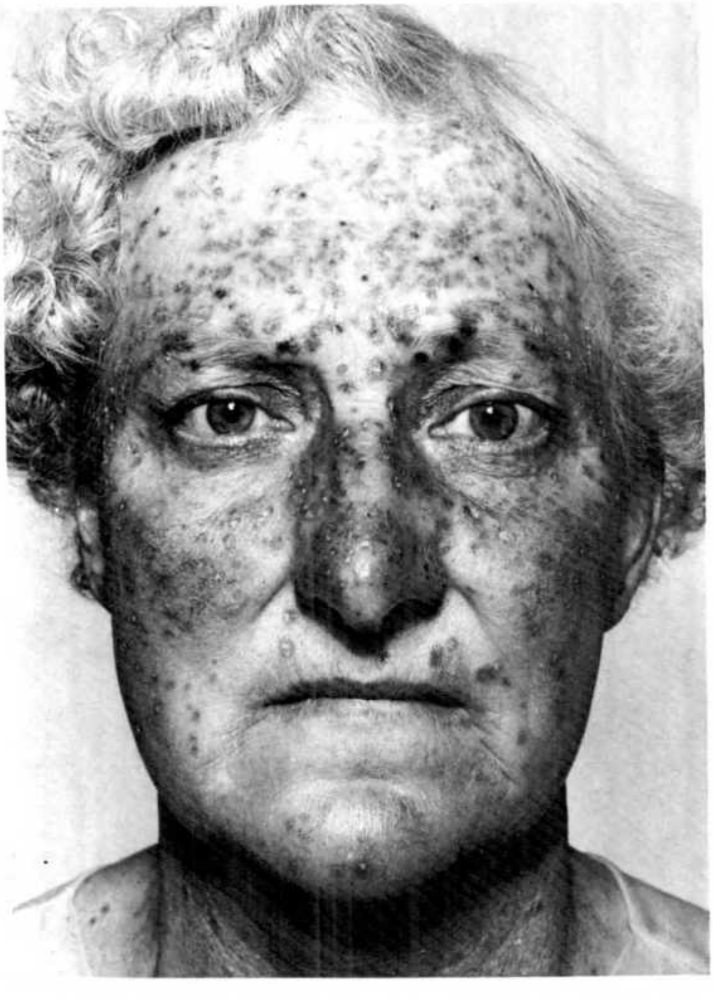

FIGURE 1. Semi-translucent papules, with superficial scaling, on the face.

In January, 1971, the rash spread to involve the neck, arms and legs. Her appetite was poor, and she had lost 13 kg in weight during the previous 6 months. She had a persistent unproductive cough for which she took Linctus of codeine. She had no dyspnoea.

Biopsy of skin—suggestive of sarcoidosis. Kveim test—positive. Mantoux, 1 in 10,000 positive. Isoniazid 100 mg three times daily commenced.

In March, 1971, she developed bruising on the hands, arms and legs, associated with recurrent epistaxis, May, 1971, all drugs were stopped. There was no improvement in her condition.

She developed swelling of the parotid glands in June, 1971 and was transferred to Cardiff Royal Infirmary for investigation.

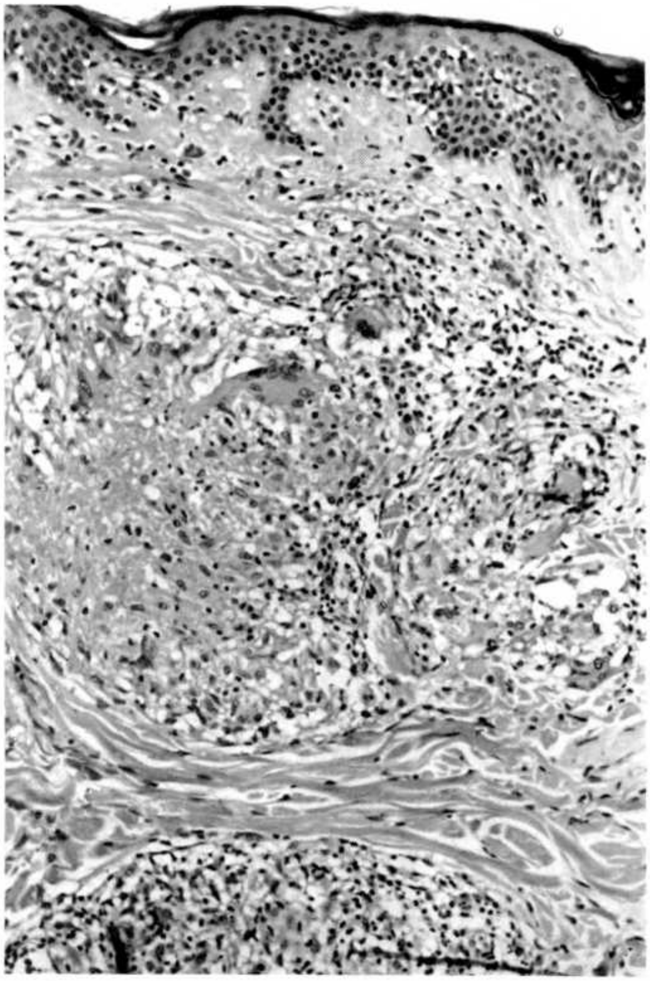

FIGURE 2. Skin biopsy of the left arm showing well defined giant cell and epithelial cell granulomata in the dermis with minimal caseation (× 123).

Examination, June, 1971. Skin. There was a widespread eruption on the face, neck, chest and arms of pale brown semi-transluscent papules with superficial scaling (Fig. 1), numerous ecchymoses on the arms and legs, and submucosal haemorrhages on the soft palate. The lymph nodes in the submandibular, anterior and posterior triangle of neck, and inguinal regions were enlarged. The liver was palpable one finger breadth below the costal margin. The spleen was not palpable. *Ophthalmic examination* (Mr Peter Graham). There was no evidence of active ocular sarcoidosis but there was a considerably reduced lacrimal secretion, not unexpected in a patient with a history of probable sarcoid involvement of the salivary glands. *Examination of other systems—no abnormality detected.*

Investigations. Hb 12.3 g/100 ml. WBC 3200/mm^3. Platelets 26,000/mm^3. 60% polymorphs, 20% lymphocytes, 20% monocytes. ESR 25 mm/h (Westergren). Marrow examination—smear showed normal cytology. Marrow section showed normal haemopoiesis, no reduction in megakaryocytes, and no abnormality to account for thrombocytopenia. Serum iron—42 μg/100 ml. TIBC 420 μg/100 ml. Bleeding time 6 min (normal up to 5 min). Clotting time 8 min (normal 5–10 min). Platelet survival ($T\frac{1}{2}$ ^{51}Cr-labelled platelets) 20 h (normal 9–11 days). Tests for isoniazid, codeine and oxytetracycline dependent platelet antibodies, by clo tretraction and agglutination, were negative. Platelet agglutination test—positive at 1:4 dilution only. Antinuclear factor test—negative. LE cell test negative. Immunoglobulins normal. Serum calcium normal. *Skin biopsy. Left arm* (Fig. 2). Numerous well defined giant cell and epithelioid cell granulomata in the dermis with minimal caseation, compatible with sarcoidosis. *Culture* for tubercle bacilli from biopsy specimen was negative. *Chest-X-ray.* Diffuse opacities seen throughout both lung fields with an enlarged node at the right hilum. *X-ray of hands.* No evidence of bony sarcoid. *Abdominal X-ray.* No evidence of splenic enlargement. *Lung function studies.* Carbon monoxide transfer test (single breath method) 16 ml/min/mmHg (Normal 21 ml/min/mmHg).

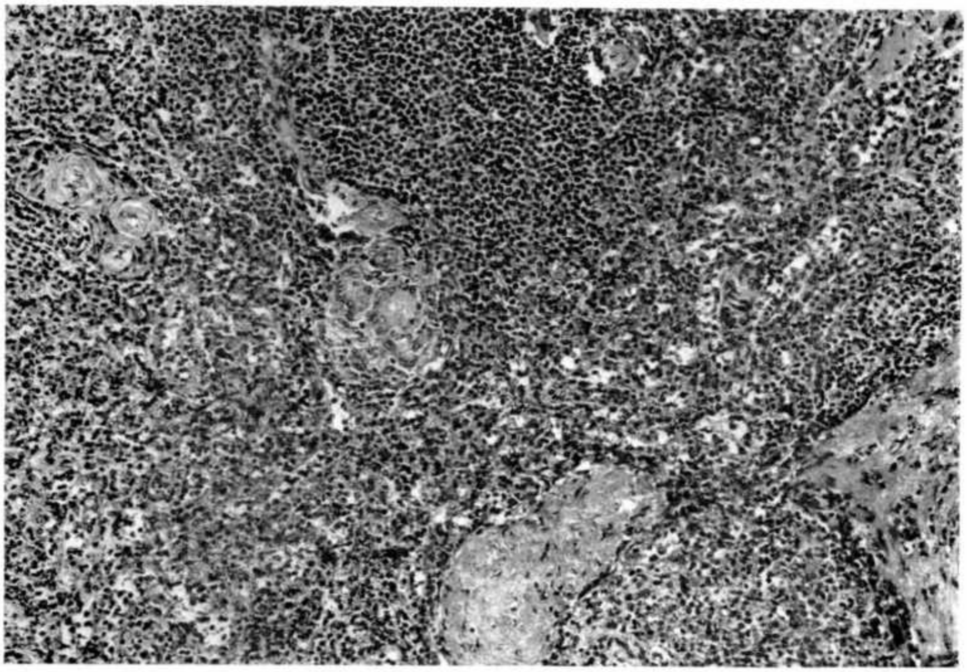

FIGURE 3. Section from spleen showing sparse, poorly formed sarcoid granulomas and prominent hyaline thickening of small vessels (× 121).

Treatment and progress. Treatment with prednisone 20 mg daily was commenced, and 4 weeks later the dosage was increased to a maximum of 60 mg daily for 2 weeks. There was no rise in the platelet count, and she had several episodes of spontaneous epistaxis. She developed steroid induced diabetes mellitus. The dose of prednisone was reduced, and, because of the failure of the steroids, splenectomy was performed (26 August 1971). A spleen weighing 285 g was removed.

Histology of spleen (Dr Jones-Williams). Multiple sections show very sparse sarcoid granulomas, poorly formed, many consisting only of giant cells (?) regressing. Hyaline thickening of small vessels is prominent. The pulp is hyperplastic and contains many polymorphs; there is no amyloid (Fig. 3).

On the second post-operative day the platelet count was 75,000/mm^3 rising to 255,000/mm^3 by the tenth day. Platelet count was 150,000/mm^3 at the time of her discharge on the eighteenth post-operative day. Treatment on discharge was 10 mg prednisone daily.

November, 1971. Platelet count 65,000/mm^3. No history of abnormal bleeding. Skin lesions now beginning to resolve. Prednisone 10 mg daily continued. Chest X-ray—'The previous pulmonary involvement has now cleared'.

February, 1972. Platelets 55,000/mm^3. She did not complain of any spontaneous bruising or bleeding. Scars on the face were fading. Prednisone stopped.

February, 1973. Platelet count 50,000/mm^3. Skin lesions healed.

Discussion. In sarcoidosis, though involvement of the spleen is common (Scadding, 1967a), thrombocytopenic purpura is very rare. Kunkel & Yessner (1950) collected seven cases of splenic sarcoid and thrombocytopenia, and Scadding (1967b) briefly summarizes nine further case reports, but there is no clear evidence suggesting a causal relationship. Kunkel & Yessner (1950) suggested that sarcoid foci in the spleen may mediate production of some agent responsible for inhibiting platelet production by the bone marrow. Edwards, Wagner & Krause (1952) and Schrijver & Schillings (1952) postulated that 'hypersplenism' may be the cause of thrombocytopenia, due either to some platelet reducing substance produced by the spleen or to enhanced phagocytosis and sequestration by an enlarged spleen, but their definition of hypersplenism was not stated. Palpable splenomegaly is held by most authorities to be one of the cardinal features of hypersplenism, but in the reported cases the largest spleen was only 680 g (a two finger-breadth enlargement), and in many of the cases the spleen was not enlarged. We could find only one or two cases where thrombocytopenia could be explained on the basis of hypersplenism as we understand it, and in most of the reported cases some other mechanism for thrombocytopenia must be invoked. If there is a causal relationship between splenic sarcoid and thrombocytopenia, then we would expect some degree of thrombocytopenia to be found in many cases with splenic involvement, but this is not so.

In our patient, the finding of plentiful megakaryocytes in the marrow and reduced platelet survival supported peripheral destruction of platelets, rather than failure of platelet production, as the mechanism of thrombocytopenia. The spleen was never palpably enlarged, and the patient benefitted from splenectomy. The slightly low white cell count found on admission rose to normal before any treatment was started, and the LE cell test was negative. The platelet antibodies in the pre-operative serum specimens are of uncertain significance; in particular we do not know whether or not they are auto-antibodies. The thrombocytopenia does not appear to be drug induced. Attempts to demonstrate drug dependent platelet antibodies *in vitro* using isoniazid, codeine and tetracycline have been unsuccessful, and withdrawal of these drugs had no effect on the platelet count.

We believe that in our case the haemostatic failure was due to idiopathic thrombocytopenic purpura co-existing with sarcoidosis.

Sarcoidosis and idiopathic thrombocytopenic purpura are common conditions, and in both the spleen is very often not palpable. The histology of the spleen in idiopathic thrombocytopenic purpura does not show any specific diagnostic features, and the diagnosis can be made only by exclusion of other recognized causes of thrombocytopenia due to peripheral destruction of platelets. In our study of the reported cases of thrombocytopenia with splenic sarcoidosis, we found that megakaryocyte hyperplasia, thrombocytopenia, and improvement after splenectomy was the rule. We therefore suggest that nearly all the reported cases may well have been idiopathic thrombocytopenia co-existing with splenic sarcoid. The evidence to date does not indicate a causal relationship between splenic sarcoid and thrombocytopenia.

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(21) Dermatitis herpetiformis with tongue lesions

DR G.C. MUSTON AND DR G.A. HODGSON

Department of Dermatology, University Hospital of Wales, Cardiff

W.C., male, aged 49 years. Coal miner.

History. The patient complains of a recurrent, itching rash on the back, arms, legs and neck for 7 years. There are grouped gyrate erythematous areas with vesicles (Fig. 1), which responded initially to treatment with dapsone 100 mg daily. 2 months later, after 2 weeks without dapsone, he developed irritation and shallow ulcers on the dorsum of the tongue (Fig. 2).

Examination. There is scarring of tongue and pigmentation and scarring on limbs and trunk.

Investigations. Blood. Full blood count, ESR, folic acid, immunoglobulins and plasma protein electrophoresis, serum complement were normal. WR, Kahn and Reiter protein complement fixation tests were negative. *X-rays.* Barium meal normal.

Histology. Skin biopsy. Sub-epidermal bulla with acute inflammation and eosinophils in the exudate (1968). *Tongue biopsy* (November 1966). Sub-epithelial non-acantholytic bullae. The cavities of the bullae and the upper layers of the dermis show a polymorphonuclear infiltrate—neutrophils and not eosinophils. Plasma cells and oedema between the collagen fibres are further features. Some bullae are intraepithelial from regeneration of epithelium at the base (Fig. 3). Accepted as dermatitis herpetiformis. *Jejunal biopsy.* Normal.

Course and treatment. Although the skin lesions were controlled by dapsone 400 mg daily, the tongue lesions were not. These seemed to improve, in contrast to the skin lesions, only when the dose was reduced. Prednisone 5 mg twice daily over a period of 3 months did not help the skin or tongue lesions. Sulphapyridine improved the skin but not the tongue. Ultimately a dose of dapsone 200 mg daily kept skin lesions at a minimal level and reasonably comfortable. A gluten-free diet did not help.

Comment. Clinical and histological findings, and the response to treatment, substantiated a diagnosis of dermatitis herpetiformis. This disorder is rare, and is more frequent in males. An association with thyroiditis and pernicious anaemia could suggest that it is an auto-immune disorder, but circulating antibodies to skin have not been described (Fraser, 1970).

Many cases, such as this, with no evidence of malabsorption, may still show subtotal or partial 'villous atrophy' on jejunal biopsy: moreover, one third of all patients have histologically and functionally normal intestinal mucosa, as in this case. Other parameters of malabsorption are also usually normal. Low serum folate levels are most often found.

Tolman, Maschella & Schneiderman (1959) stated that mucous membrane lesions are absent in dermatitis herpetiformis, but cases have been reported (Cooke, 1970; Russotto & Ship, 1971). In general, oral lesions appear to be rare and are usually mild and transient, tending to appear after the skin lesions have developed. Exceptionally, they may be the first manifestations (Pindborg, 1968).

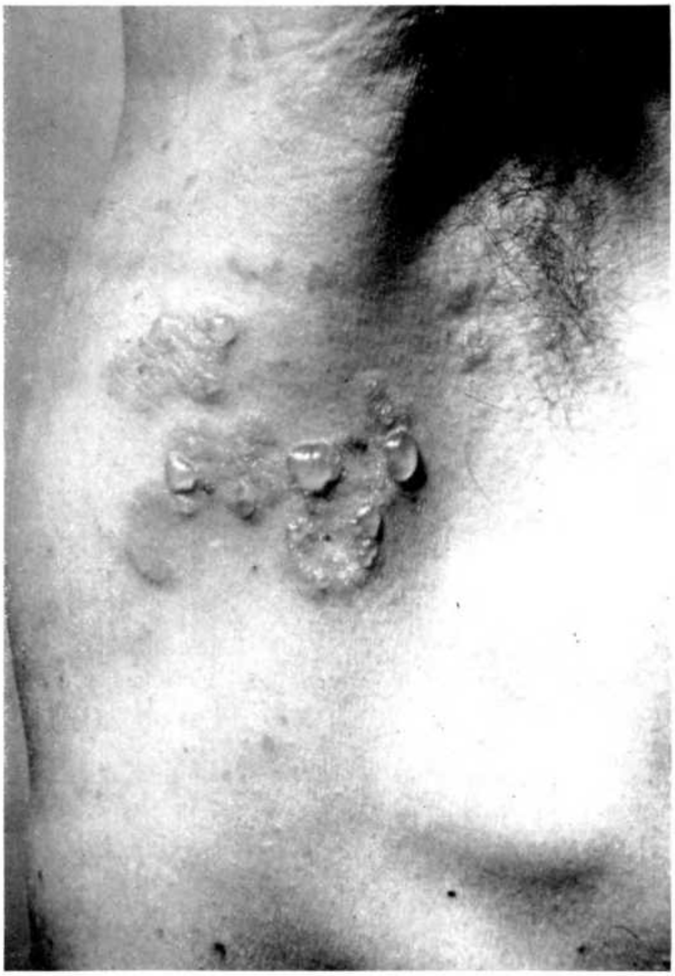

FIGURE 1. Vesicular axillary lesion.

Control of the disorder in this case needed a high dosage of dapsone, but as this seemed to aggravate the tongue lesions a compromise smaller dose has proved acceptable. The serum levels of dapsone after a single oral dose, such as is used for treatment, vary considerably in control patients and those with dermatitis herpetiformis, but its absorption is uninfluenced by the presence or absence of jejunal

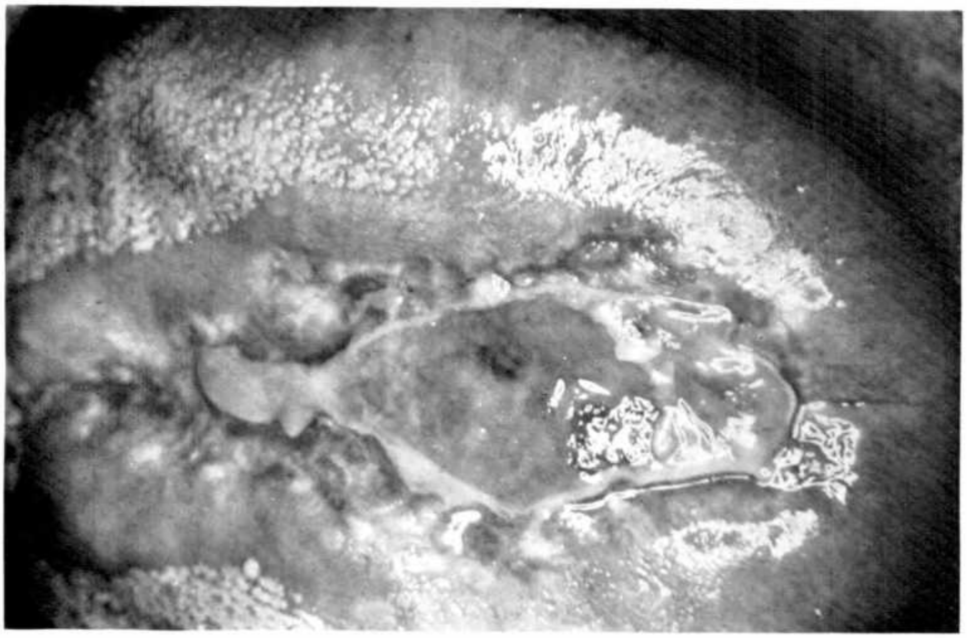

FIGURE 2. Tongue lesions of dermatitis herpetiformis.

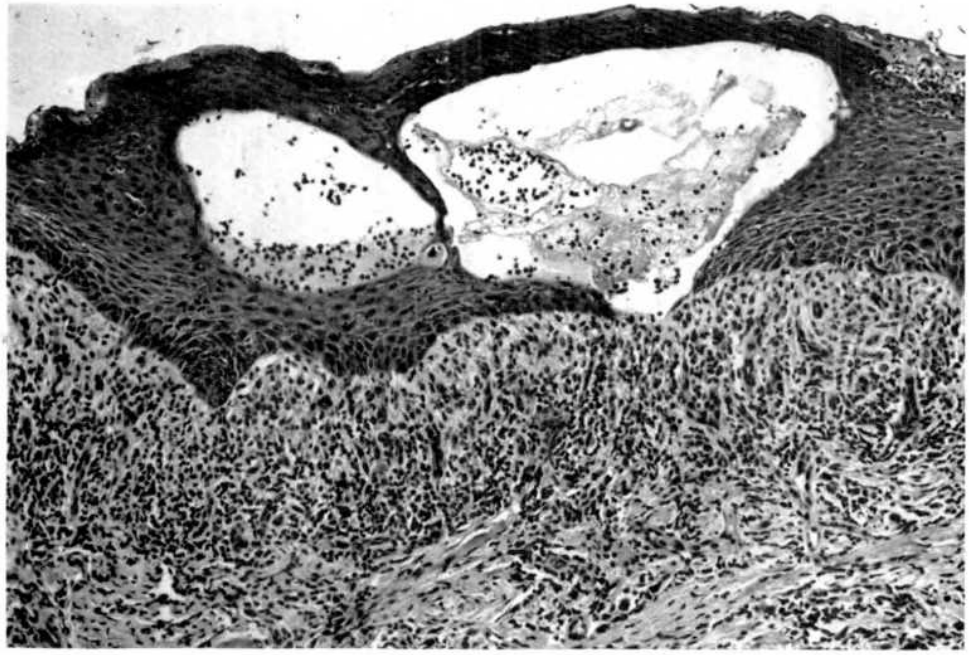

FIGURE 3. Tongue biopsy showing bullae and polymorphic neutrophil infiltrate with plasma cells and oedema between collagen fibres (× 89).

abnormality (Alexander *et al.*, 1970). Symptomatic improvement after withdrawal of gluten from the diet has been reported (Marks & Whittle, 1969) in many cases, but here it had no effect, and was abandoned by the patient as unacceptable.

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(22) Juvenile pemphigoid

DR A. CATTELL AND DR E. WADDINGTON

Department of Dermatology, University Hospital of Wales, Cardiff

H.E., female, aged 8 years.

History. In April 1972, 2 weeks after acute tonsillitis, she developed 'ulcers' in the mouth and pink spots on the knees and buttocks; these became vesicular within 48 h. During the next 10 days she

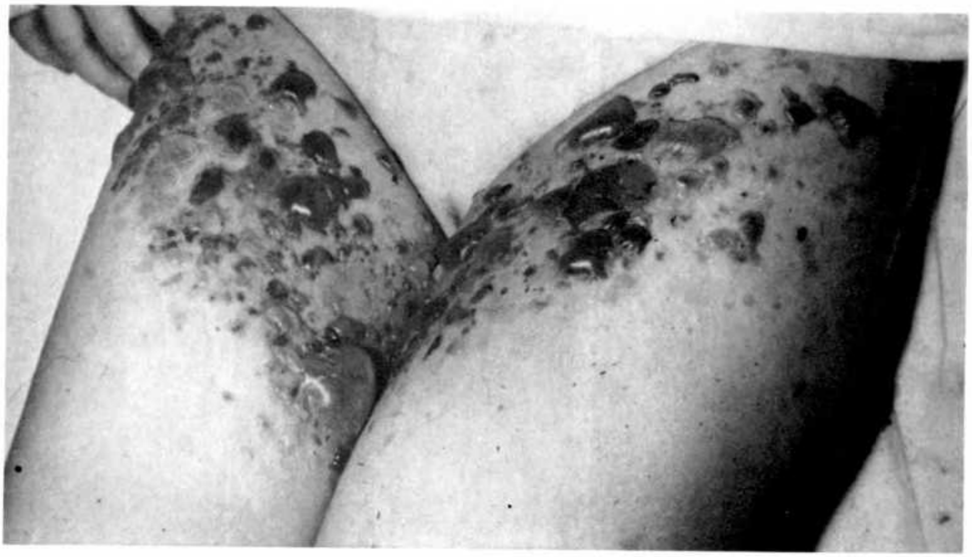

FIGURE 1. Large haemorrhagic bullae at a characteristic site.

developed a severe widespread rash consisting of blotchy red patches and large blisters. She was not ill and had little irritation.

Examination. The eruption was widespread. The lesions consisted of both clear and haemorrhagic bullae, some arising on normal skin (Fig. 1), but the majority were situated on polycyclic erythematous patches (Fig. 2). The most severe lesions affected the upper thighs, genitalia, buttocks, upper arms, axillae, perioral area and neck.

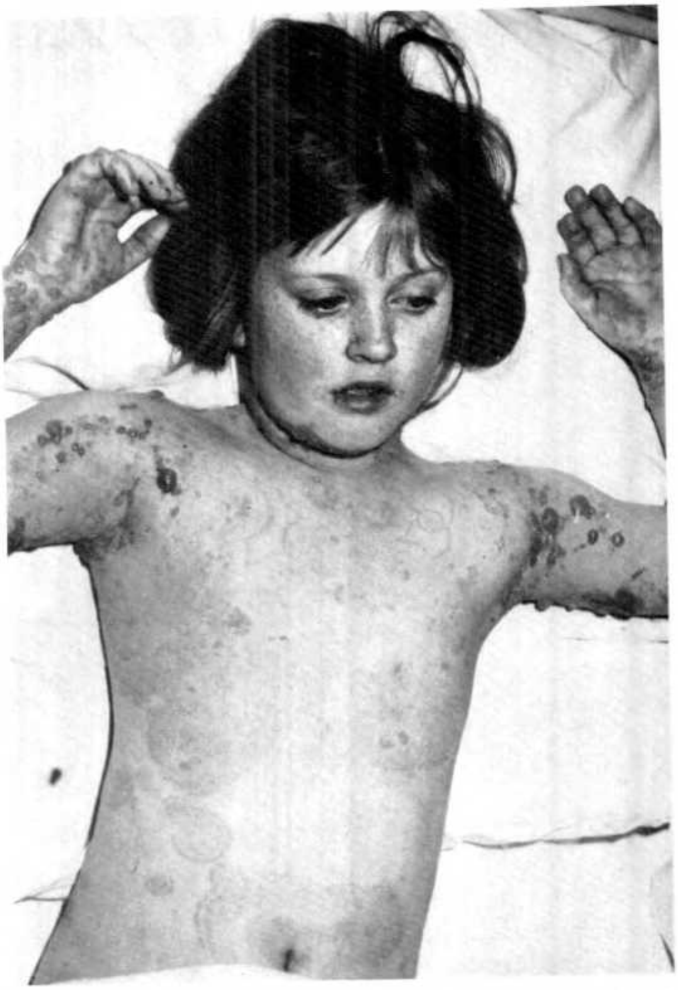

FIGURE 2. Early stage showing the polycyclic erythematous patches which precede the formation of bullae.

Course. During the first week after admission new gyrate erythematous plaques developed on the trunk, and bullae arose along their margin. The lesions eventually affected most of the skin surface, including fingers, palms, soles, pinnae, eyelids, nasal vestibules, lips, buccal mucosae, tongue and palate.

Investigations. Hb 12·8 g/100 ml. WBC 19,100/mm^3. Normal differential count. Immunoglobulins, liver function tests, blood urea and electrolytes, xylose tolerance, all normal. Indirect immunofluorescence—negative.

Histology of skin (Fig. 3). There is a zone of polymorph infiltration immediately under the epidermis, with the formation of subepidermal bullae and vesicles containing fibrin, polymorphs and occasional eosinophils. The deeper parts of the dermis are normal.

Jejunal biopsy. Normal.

Treatment. Sulphapyridine 500 mg twice daily produced severe vomiting, and was stopped after 5 days. The eruption became more widespread during this period and prednisone 40 mg and dapsone 100 mg daily were commenced. There was a dramatic improvement. A gradual reduction in prednisone dosage was made. She is now receiving prednisone 3 mg and dapsone 50 mg daily. Frequent relapses have occurred, but none as severe as the original attack.

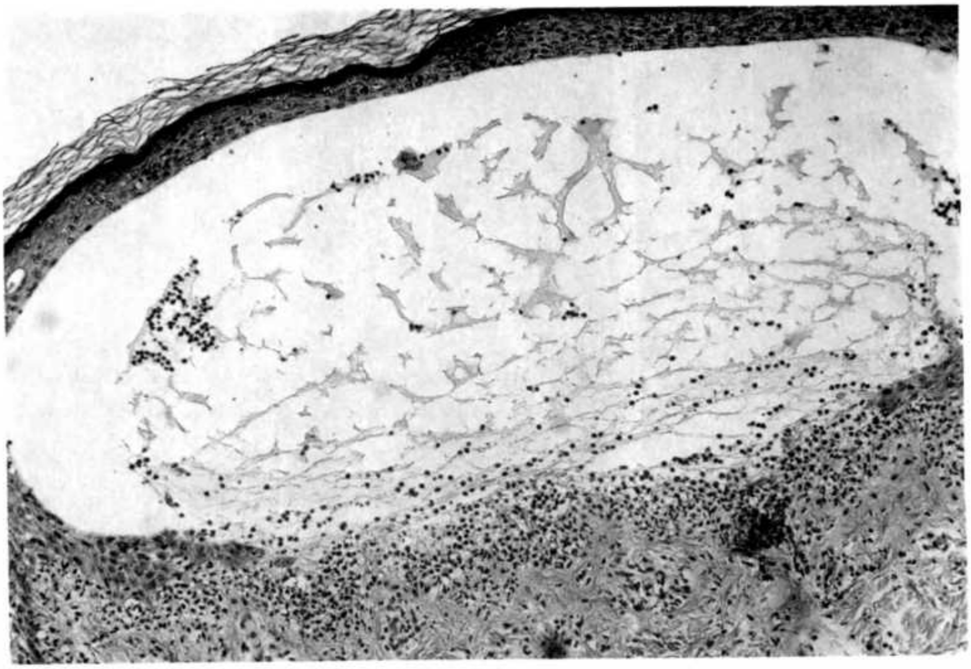

FIGURE 3. Subepidermal bulla containing fibrin, polymorphs and eosinophils (× 95).

Comment. The treatment has been difficult because of intolerance to sulphapyridine. She has had an unusually widespread eruption with involvement of the palms, soles and oral mucosae. Kim & Winkelmann (1961) reviewed twenty-two cases, seen over a period of 40 years. The oral mucosa was involved in one patient and the palms and soles were never involved. In Grant's (1968) series of thirty-eight cases, only one child had oral lesions.

Immunofluorescence studies have produced conflicting results. Jordon *et al.* (1970) were unable to demonstrate basement membrane antibodies in six children. Jablonska *et al.* (1971) studied eight patients; three showed the immunological features of bullous pemphigoid, two those of dermatitis herpetiformis and three were negative. These authors therefore concluded that juvenile pemphigoid is not a nosological entity.

The clinical picture in this patient closely resembles bullous pemphigoid and is quite unlike dermatitis herpetiformis.

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(23) Cicatricial pemphigoid

DR G.C. MUSTON AND DR E.WADDINGTON

Department of Dermatology, University Hospital of Wales, Cardiff

E.D., male, aged 62. Retired chartered accountant.

History. In 1962 he developed recurrent 'hard blisters' in the mouth which persisted for 2–3 days before they ruptured, leaving ulcers. They have affected the tongue, palate, buccal mucosae and lips. buccal mucosae; numerous erosions covered with fibrinous sloughs affected the palate and under surface of the tongue. There was a purulent gingivitis. There were no skin or conjuctival lesions.

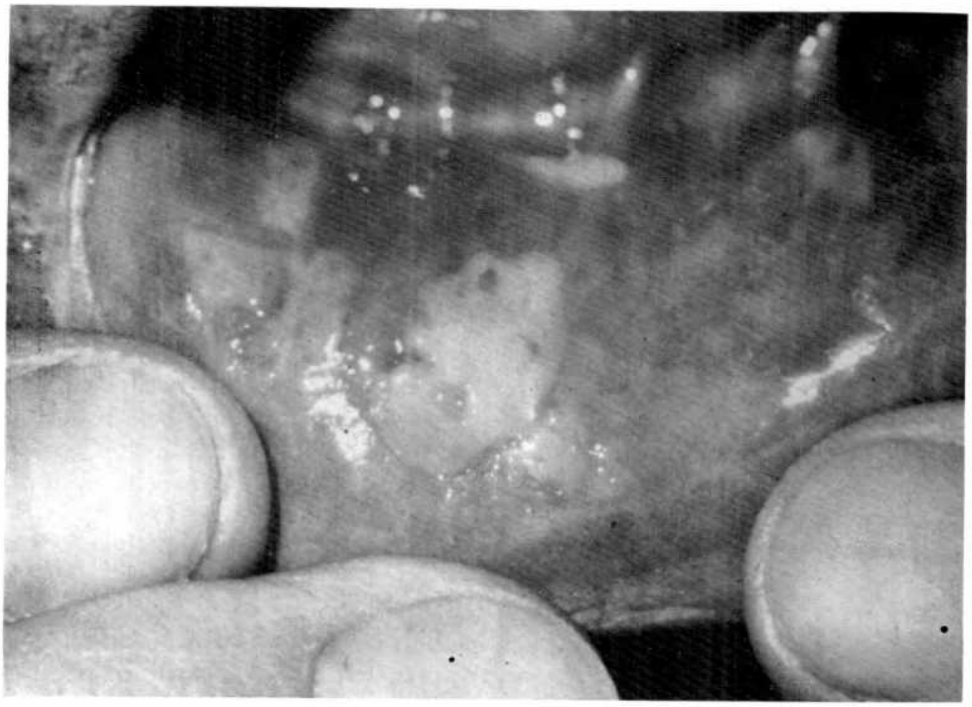

FIGURE 1. Ruptured bulla on mucosa covered with fibrinous slough.

On examination (Fig. 1). In 1964 there were tense bullae, one of which was haemorrhagic, on the
Investigations. A biopsy from the mucosa showed a subepithelial bulla. A barium swallow showed a marked post-cricoid impression.

Course. 6 months after he was first seen in 1964, both clear and haemorrhagic bullae developed on the elbows, knuckles, palms and right upper eyelid. These lesions disappeared in a week or two, but bullae recurred on the elbows at intervals of a few months for about 1 year. *Skin biopsy* shows a subepidermal bulla which is filled with fibrin and scanty polymorphs. There is minimal inflammatory infiltrate in the dermis. There is some regeneration of squamous epithelium along the floor of the bulla (Fig. 2). He has had no skin lesions since 1965 but has had recurrent oral lesions giving rise to erosions and scarring. In 1971 he had noticed stickiness and soreness of the eyes. No evidence of conjunctival adhesions. He also developed increasing difficulty in swallowing solids. In 1972 a barium swallow showed an extensive irregularity in the pharynx, with narrowing of the lumen, which suggested a neoplasm (Fig. 3). *Oesophagoscopy.* There was a large bulla in the post-cricoid area. The superficial layer was ruptured and detached. Below this there was a tight stricture 1 cm in diameter with considerable fibrosis. The stricture was dilated, but attempts to pass the oesophago through were abandoned because of haemorrhage.

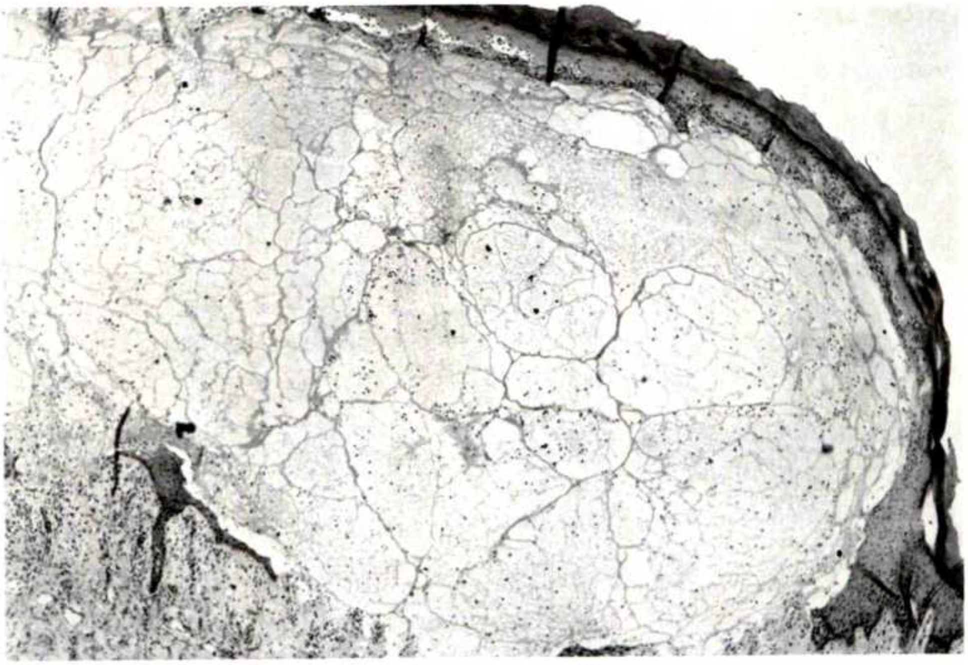

FIGURE 2. Biopsy shows a subepidermal bulla, minimal dermal infiltration and some regeneration on the floor of the bulla (× 33).

Treatment. He was initially treated with prednisone (80 mg daily), the dose gradually, being reduced over the course of 6 months. This produced a remission, but bullae recurred in the mouth a few months after stopping treatment. They were treated with topical steroid. In 1972, when the oesophagus became involved, systemic prednisone was reintroduced, and he remains symptom-free on a dose of 3 mg daily. Oesophagoscopy in September 1972 showed no lesions in the hypopharynx.

Comment. Despite the 11-year history, this man has fortunately not developed ocular scarring. The oesophagus became involved 9 years after the onset. Corticosteroid therapy in low dosage appears to have produced a remission of the oesophageal bullae and stricture, although it has only had a slight beneficial effect on the oral lesions.

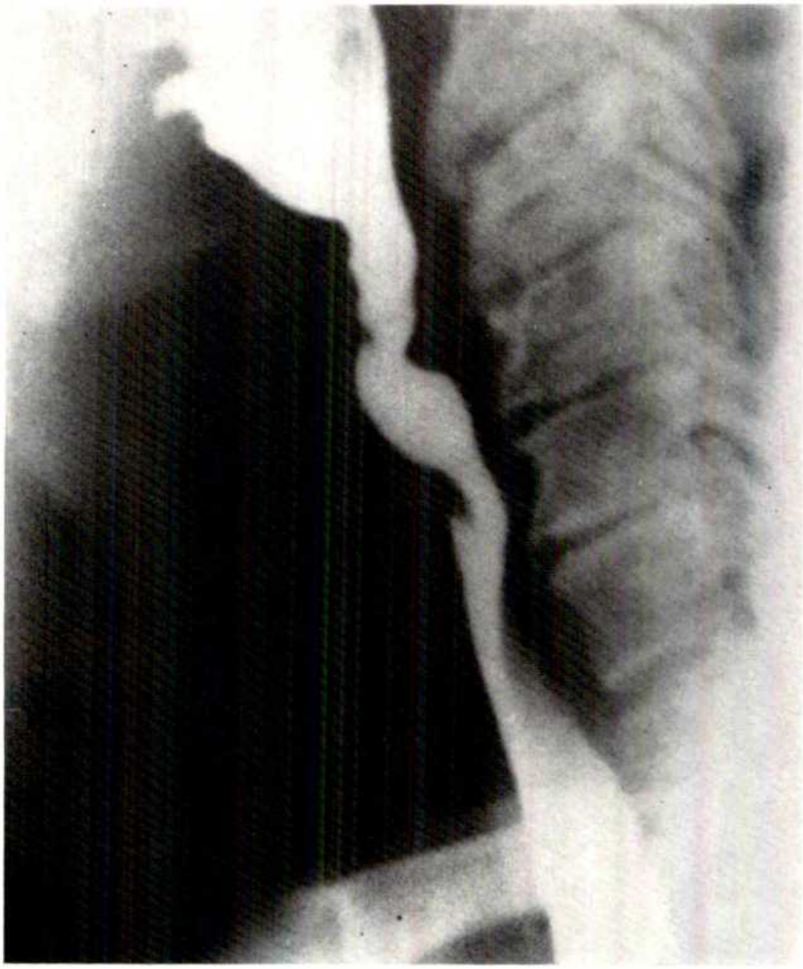

FIGURE 3. Barium swallow demonstrating the extensive irregularity with narrowing of the lumen.

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(24) Pemphigus vegetans

Dr A. J. Reynolds and Dr E. Waddington

Department of Dermatology, University Hospital of Wales, Cardiff

J.P., male, aged 58 years. Carpenter.

History. In June, 1964, he presented with a 4-month history of recurrent epistaxis, crusting in the nostrils and ulceration of the lips, buccal mucosae and palate. During the last 2 months, the tip of his nose had become swollen and red with a few scattered papules resembling rosacea. There was no history of diarrhoea.

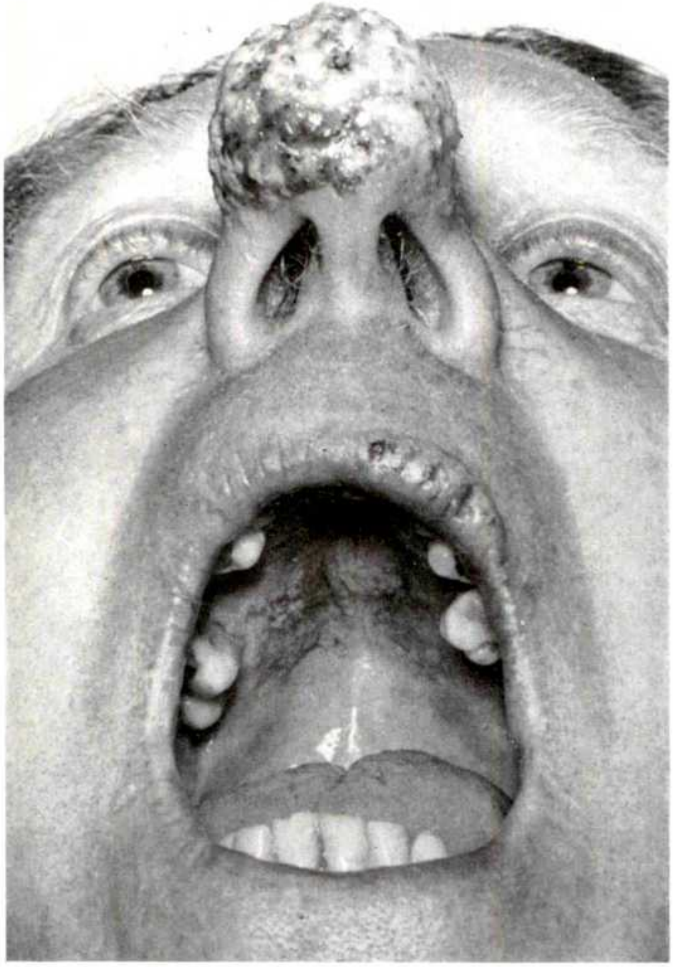

FIGURE 1. Purulent mass on the nose. Less florid vegetations on the lip and palate.

Examination. There were scattered erythematous papules and pustules on the tip of the nose which later coalesced to form a purulent vegetating mass (Fig. 1). There were grossly infected vegetating lesions on the mucous membranes of the mouth and in the nasal vestibules (Fig. 2). There was a small vegetating nodule on the left side of the upper lip. No evidence of ulcerative colitis.

Investigations. Tzanck smear. Many eosinophils, and groups of epithelial cells showing varying degrees of acantholysis. *Biopsy of palate* (Figs. 3 and 4). Epithelial hyperplasia with the development of a suprabasal vesicle containing polymorphs, eosinophils and acantholytic cells. The dermis contains a heavy inflammatory infiltrate, mainly of plasma cells with occasional eosinophils. *Biopsy of nose (skin).* Epithelial hyperplasia and acanthosis with polymorph accumulation in a hair follicle shaft. Numerous eosinophils in the dermal infiltrate.

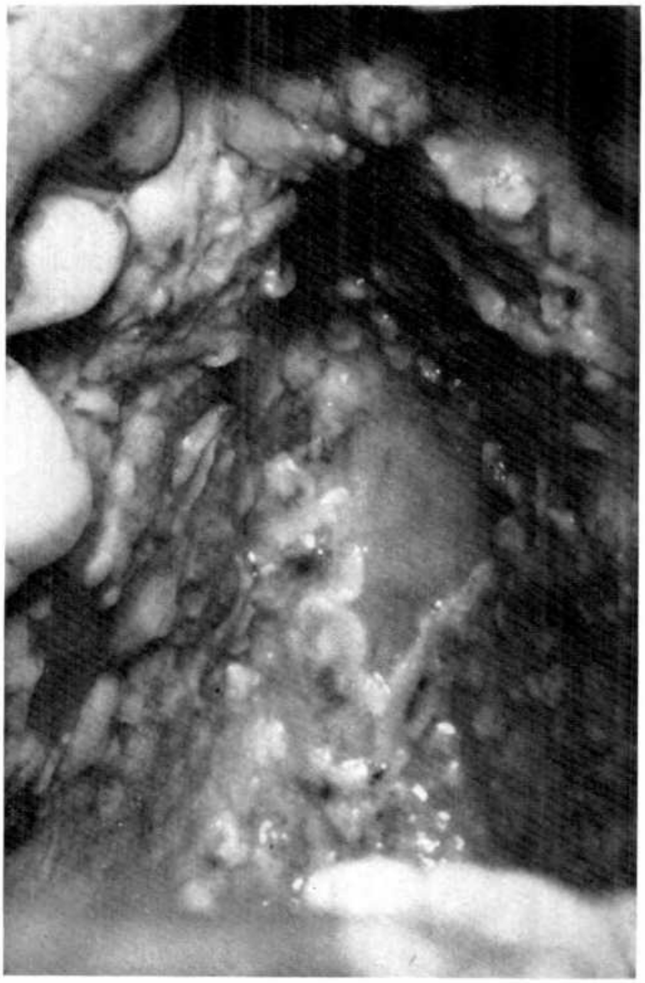

FIGURE 2. Severe relapse. Infected vegetations on the palate.

Course and treatment. Prednisone 100 mg daily rapidly controlled the lesions. Oral steroids were discontinued in October, 1965. 1 year later there was a relapse. During the last 8 years he has required prednisone 5–15 mg daily. The disease has been in remission again since February, 1973, and prednisone therapy has been stopped.

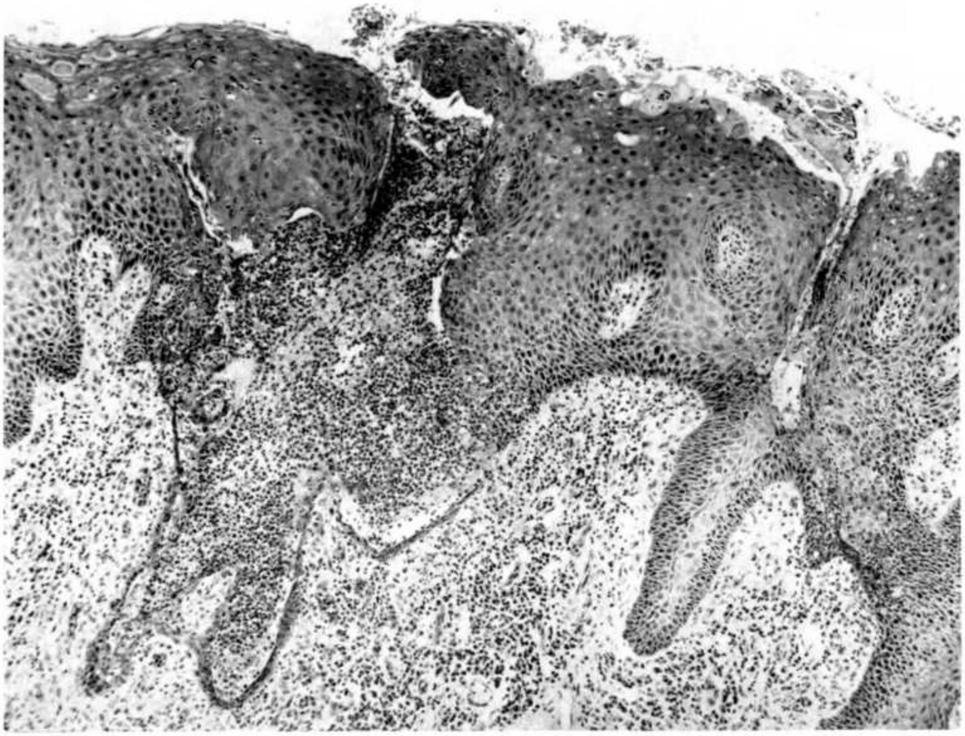

FIGURE 3. Biopsy of palate showing suprabasal cleft.

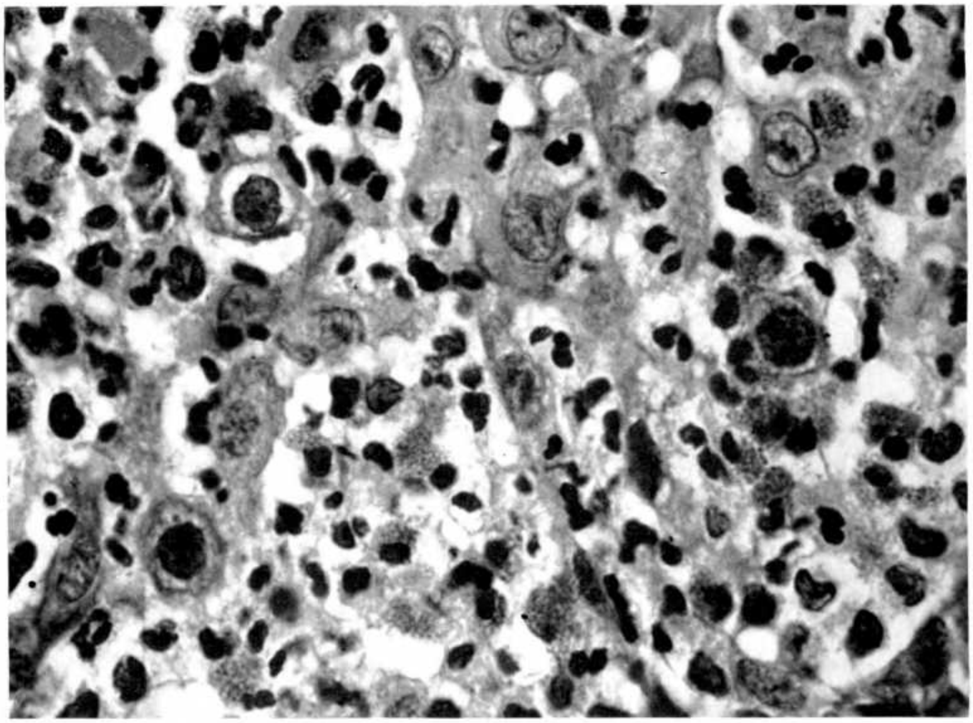

FIGURE 4. Acantholytic cells in the intra-epidermal vehicle.

Comment. This patient's lesions have been restricted to the buccal and nasal mucosae, the lip and the tip of the nose. The grossly infected vegetations in the mouth and nose suggested the Hallopeau type of pyodermite végétante, but the histology is typical of pemphigus vegetans. He has no evidence of ulcerative colitis, which may be associated with the Hallopeau type (Forman, 1966), nor is there any evidence of a malignant neoplasm, which has recently been reported in association with pemphigus (Saikai & MacConnell, 1972).

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(25) Porphyria cutanea tarda symptomatica

DR D.R.W.DAVIES AND DR G.A.HODGSON

Department of Dermatology, University Hospital of Wales, Cardiff

J.McM., male, aged 48 years.

History. In June, 1972, a recurrent blistering was first noticed on the dorsa of both hands overlying metacarpophalangeal joints, and on the forehead, aggravated by friction and sunlight. At this time the skin was more pigmented than usual, and the urine was dark on voiding. There were no symptoms referrable to the abdomen or central nervous system. The patient admits to being a heavy beer drinker. 6 years ago he was treated for coronary thrombosis; liver palms and hepatomegaly were noticed then. In 1942, there was a short episode of jaundice. No positive family or drug history was obtained.

Examination. He is of ruddy complexion, with a hirsute face, pigmented on sun-exposed areas. There is scarring, but no blistering, over the metacarpophalangeal joints and forehead (Figs. 1 and 2), and there are milia on the dorsa of both hands. There are liver palms, but no other stigmata of liver disease. The liver is palpable 5 cm below the costal margin, and is firm. The tip of the spleen is just palpable. Both these findings were confirmed on liver scan.

Investigations. Hb 16·2 g/100 ml with normal film. Serum bilirubin marginally elevated (22 μmol/l). SGOT marginally elevated (65 IU/l). Kunkel, normal. Serum proteins and electrophoresis normal. Serum iron 128 μg/100 ml. TIBC 375 μg/100 ml. Glucose tolerance test normal. *Urine:* uroporphyrin 3800 μg/l (normal range 0–30); coproporphyrin 470 μg/l (normal range 0–160); porphobilinogen 1·0 μg/ml; 5-aminolaevulinate 4·0 μg/ml (normal 6·0). *Faeces:* coproporphyrin 88 μg/g dry weight (normal 27); protoporphyrin 66 μg/g dry weight (normal 75). *Liver biopsy.* Red fluorescence under ultra violet light, and low grade hepatitis with early fibrosis (Fig. 3).

Course and treatment. Venesection of 400 ml weekly carried out for 2 months. The haemoglobin decreased to 12·8 g/100 ml. Serum iron remained normal at 122 μg/100 ml. Urine uroporphyrins (at end of venesection course), 210 μg/l. He has also abstained from alcohol and as yet has had no further bullae, but remains hirsute.

Comment. Venesection, advocated by Ippen (1960), has been established as effective therapy in PCT. Clinical and biochemical improvement resulted after removal of 2·5–8·5 l blood (Epstein & Redeker, 1968). In fifteen patients improvement commenced during therapy, but in the majority there was no change in hirsutism or pigmentation for several months after treatment. Eleven patients did not reduce their alcohol intake. Saunders (1963) in South Africa, and Lundvall (1971) in Sweden, also obtained good results despite continued ingestion of alcohol. The mode of action of venesection is not clear, and excess iron in itself will not cause PCT, which is not associated with haemochromatosis (Taddeini & Watson, 1968). Lundvall (1971), however, concludes that venesection is effective through reduction of iron stores. Biochemical relapse occurred in all his patients who received further iron replenishment. In both Epstein's and Lundvall's series the initial serum iron level did not appear to be of prime importance. The main disadvantage of venesection is the inconvenience; and low

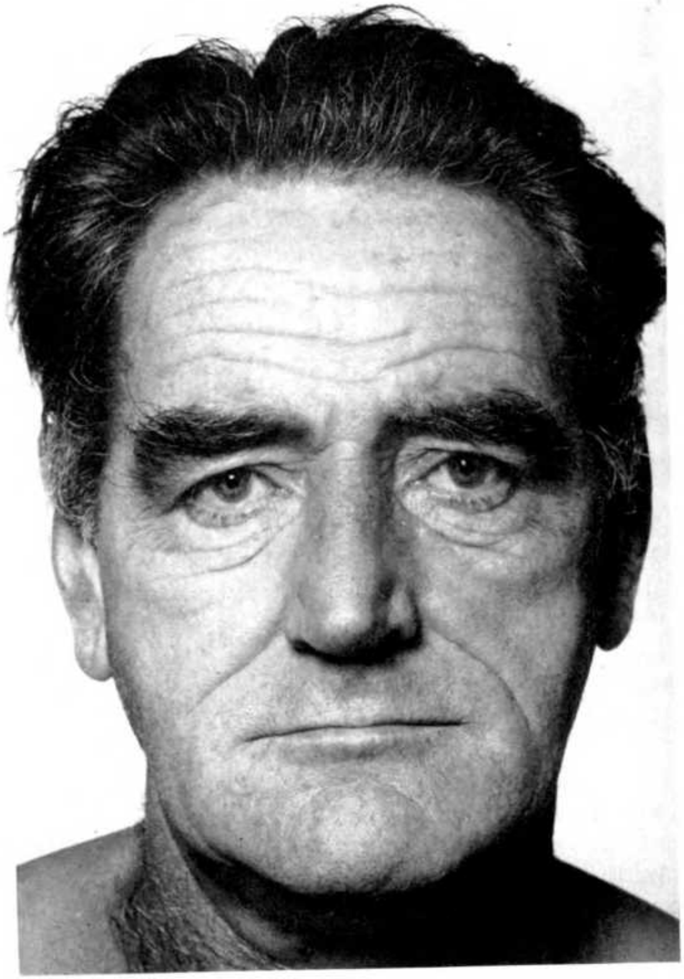

FIGURE 1. Hirsutism and scarring on forehead.

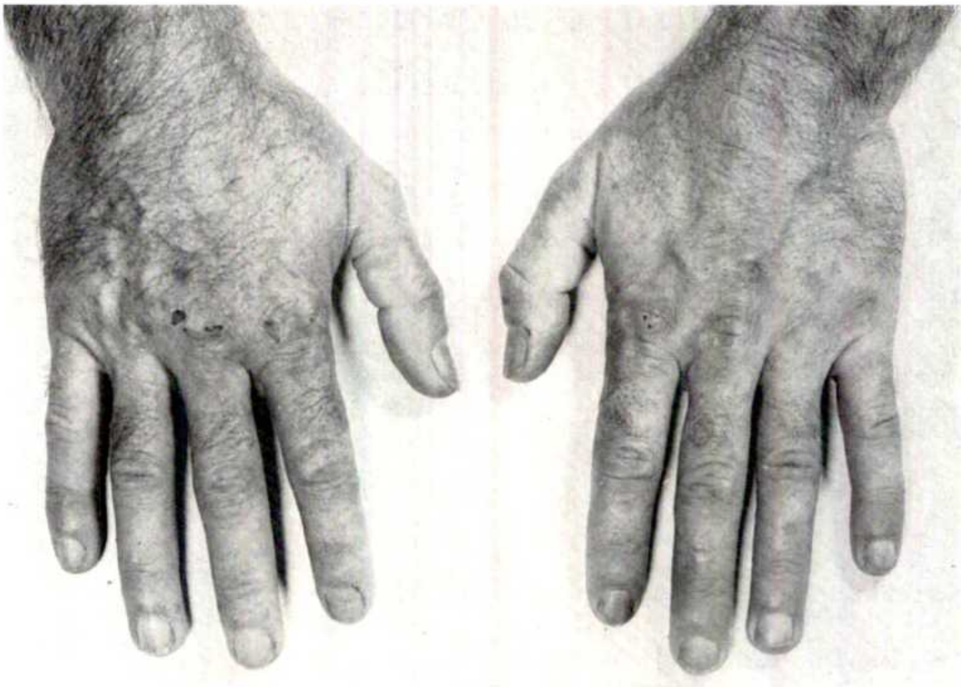

FIGURE 2. Scarring and milia of fingers.

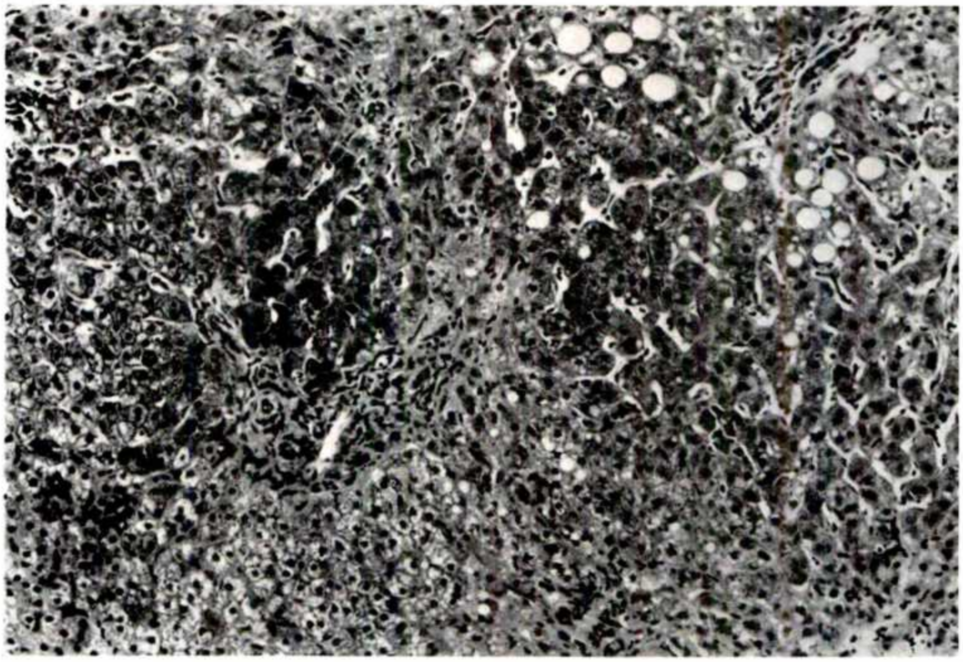

FIGURE 3. Liver biopsy: fibrotic hepatitis (× 130).

dosage chloroquine (300 mg twice weekly) has recently been found effective without the acute reactions usually associated with chloroquine (Taljaard *et al.*, 1972).

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(26) Necrobiosis lipoidica presenting on the face and scalp

Dr A. Navaratnam and Dr G.A. Hodgson

Department of Dermatology, University Hospital of Wales, Cardiff

L.E., female, aged 71 years.

History. In 1958 the patient developed on the nose and cheeks multiple papules which later subsided and then developed into red scaling horny plaques. These persisted.

In 1973 a nodule developed rapidly on an established plaque. Clinically it was a keratoacanthoma, which is now regressing after 6 months.

On examination. The patient is an obese woman with xanthelasma of both upper eyelids. There are erythematous hyperkeratotic plaques with atrophic centres and yellow scaling edges on the cheeks and forehead; there is no follicular plugging. A regressing nodule on the bridge of the nose has a central crater, like a keratoacanthoma (Fig. 1). The patient is diabetic.

Investigations. (1958). Fasting blood sugar 187 mg/100 ml and 240 mg/100 ml. (1973). Blood sugar, fasting, 180 mg/100 ml. 1½ h later 380 mg/100 ml. Liver function tests normal. Serum cholesterol 180 mg/100 ml. WR and Kahn negative.

Skin biopsy. Forehead (a) (1958). Changes compatible with necrobiosis lipoidica. Forehead (b) (1973). Changes compatible with necrobiosis lipoidica. Nose (c). Fragmented hyperplastic squamous lesion compatible with a well differentiated squamous carcinoma or a keratoacanthoma.

Course and treatment. Treatment with topical steroids and hydroxy-chloroquine (on assumption of clinical diagnosis of discoid lupus erythematosus) produced no change (1958). When seen again, the diagnosis was histologically established as necrobiosis lipoidica. The lesions have remained static, but the kerato-acanthoma is regressing and the diabetes mellitus controlled on diet and oral therapy.

Comment. Wilson-Jones (1971) confirmed the existence of an atypical annular form of necrobiosis lipoidica which is not uncommon, which has a peculiar predilection for the upper face and scalp margin in early middle life, and which is often misdiagnosed as annular sarcoidosis.

The commoner type of necrobiosis lipoidica is comparatively rare, occurring in about three per thousand diabetics (Muller & Winkelmann, 1966). Diabetes is only occasionally associated with this variant of necrobiosis lipoidica (Wilson Jones, 1971) on the face. Our patient has a mild diabetes of late onset, controlled on oral therapy and diet. It is interesting to note that in the last 6 months she has developed a nodular lesion on the nose, clinically and histopathologically suggestive of a kerato-acanthoma.

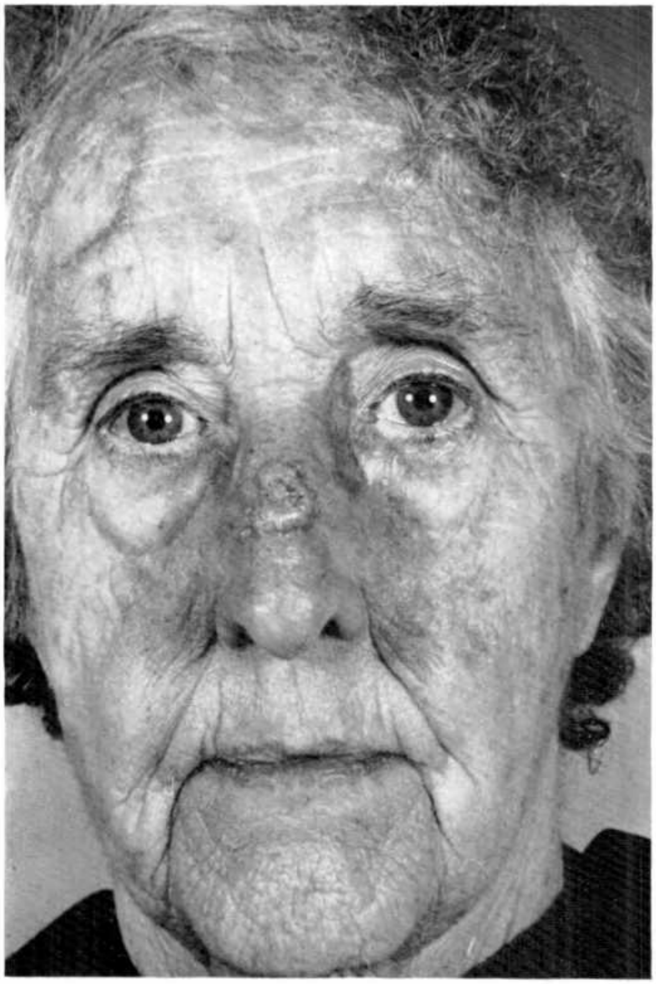

FIGURE 1. Face showing atrophic plaque on the forehead, and a clinical keratoacanthoma on the nose.

Neoplasia occurring in association with scarring areas of necrobiosis appears to be very uncommon (Muller & Winkelmann, 1966). One squamous cell carcinoma was recorded in 171 cases (Muller & Winkelmann, 1966). The regressive course of the tumour in this case suggests it is a kerato-acanthoma.

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(27) Primary self-healing squamous epithelioma of the skin

DR C. LONG

Department of Dermatology, University Hospital of Wales, Cardiff

W.P., male, aged 59 years.

History. He attended Dermatology Out-Patients in December, 1970, with an infiltrated plaque, 2·5 cm in diameter, on the right ala nasi. There was surrounding erythema with multiple visible sebaceous glands; many crusted areas approximately 2–4 mm in diameter were on the surface of the lesion, which was painful on pressure. No lymphadenopathy was found. The clinical appearance was suggestive, but not typical of a keratoacanthoma (KA) (Fig. 1).

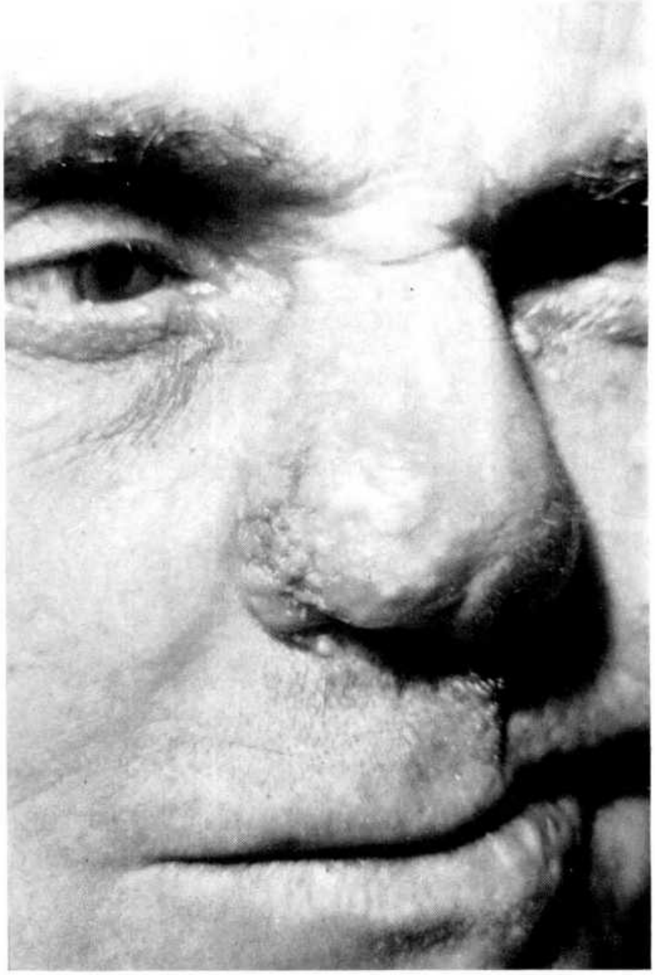

FIGURE 1. Healing stage of most recent lesion.

Histology. The histology shows localized epithelial hyperplasia with acanthosis and the formation

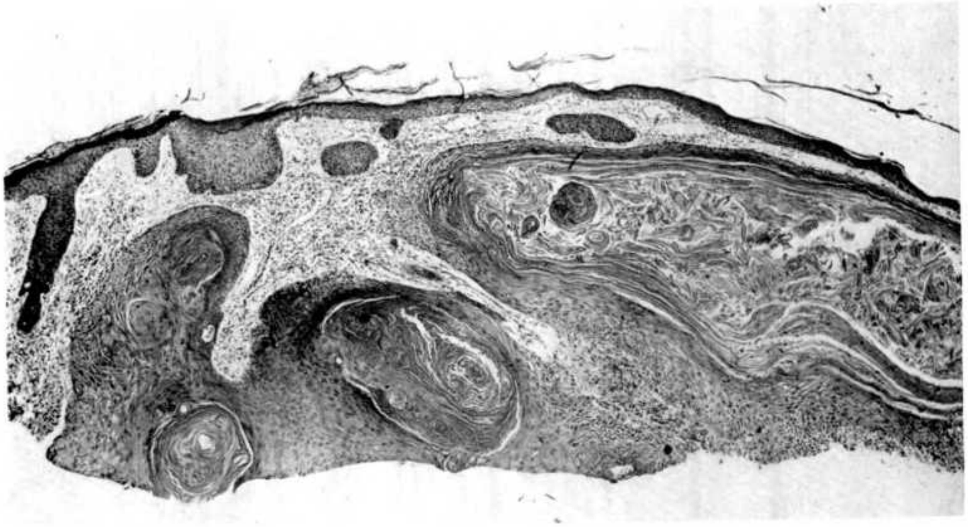

FIGURE 2. Section showing columns of hyperplasia and keratin cysts undermining epidermis (× 33).

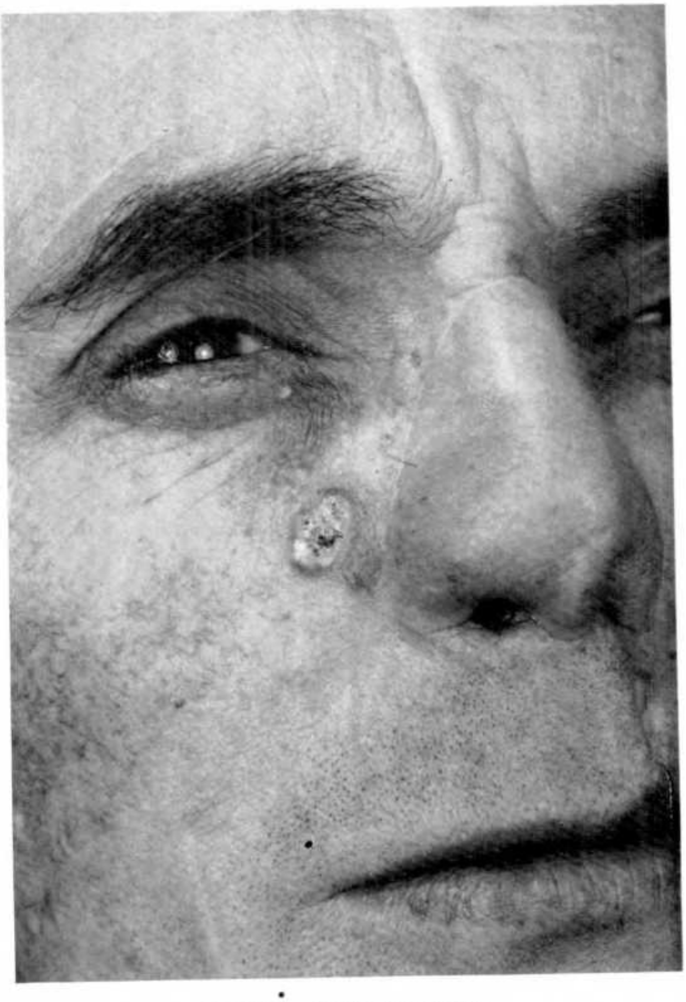

FIGURE 3. Lesion regressing.

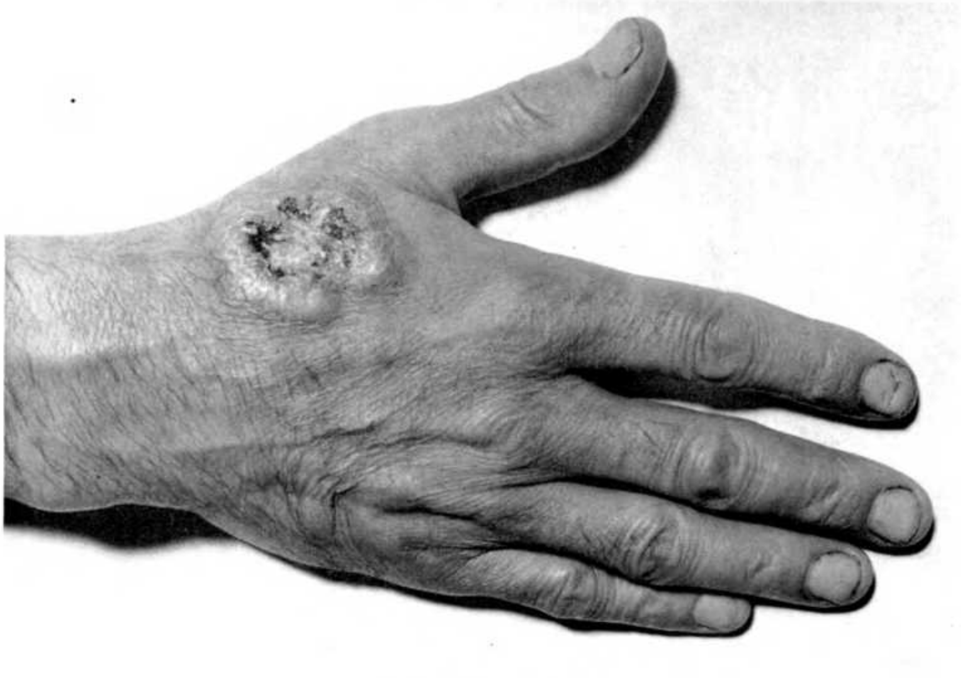

FIGURE 4. June 1963.

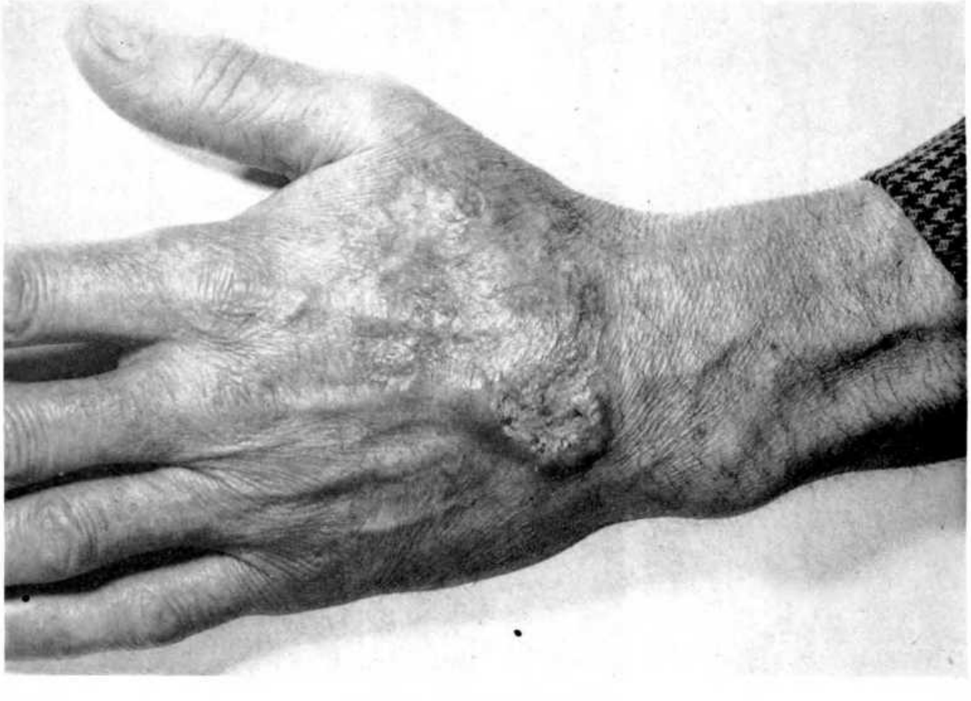

FIGURE 5. December 1966.

of keratin cysts lined with atypical epithelium. These projections extend into the dermis under apparently normal epidermis, unlike the characteristic sharp localization of keratoacanthoma (Fig. 2).

The lesion had begun in October, 1970, and eventually cleared spontaneously 6 months later. A previous history of eight other similar lesions suggestive but not typical of KA was obtained.

(1) 1950. Tip of nose. Biopsy inadequate. (2) Upper lip. *Histology*. Invasive epithelioma. Treated respectively with radium mould and superficial X-ray with no response. July, 1951. Large part of nose excised. 1953–54. Plastic surgical repair.

(3) December, 1956. Right cheek (Fig. 3). Enlarged to 2 cm in diameter in 2 weeks. Regressed in 6 weeks. *Histology*. (a) Features suggest KA rather than carcinoma. (b) Atypical epithelial hyperplasia and some chronic inflammatory exudate in the dermis.

(4) April, 1959. Dorsum of right hand. *Histology*. (a) Base is incomplete, but features are those of KA and not carcinoma. (b) Irregular hyperkeratotic lesion suggestive but not diagnostic of carcinoma.

(5) 1963. Dorsum of right hand (Fig. 4). Healed in 12 months.

(6) December, 1965. Dorsum right wrist, reaching size of 2·5 cm in diameter. *Histology*. Suggestive of squamous carcinoma. Healed in 6 months.

(7) April, 1966. Dorsum of left hand (Fig. 5). *Histology*. Section shows KA.

(8) February, 1970. Dorsum of right hand. Cleared in 6 months.

Comment. There were nine lesions in all, over a 20-year period. All lesions were clinically suggestive but not typical of keratoacanthoma. The horny crust was not complete over any of the lesions but consisted of multiple small crusts 2–4 mm in diameter. The tumours were flatter and some larger and more irregular in outline than a KA (Calnan & Haber, 1956). No family history was obtained. All lesions, except the first, cleared spontaneously.

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(28) Pitch worker's skin

DR GEOFFREY HODGSON

Department of Dermatology, University Hospital of Wales, Cardiff

T.A.C., male, aged 61 years.

History. Patent fuel marker for 40 years; high pitch exposure for 15 years. Pitch and coal are fused together. Persistent sunburning ('the smarts') and increased melanosis for years. No neoplasms for 36 years, but in the last 4 years has had two pitch warts (keratoacanthomas) (hand and face), two squamous keratoses (hand and face) and two squamous carcinomas (hand and scrotum) (1969 and 1972). No occupational exposure to petroleum oil. Smokes 20 cigarettes a day.

Family history. Father a pitch worker 40 years; no tumours.

Examination. Melanosis (poikiloderma-like on neck) with small keratoses, punctate and macular telangiectasia on hands and face. Comedones on face and back (Fig. 1).

Histology. Proven squamous keratoses and squamous cell carcinoma face (Fig. 2) and hands 1969, 1972). Scrotum; atypical hyperplasia and acanthosis with inflammatory changes to be regarded as squamous carcinoma (1972).

X-ray chest. Ill-defined opacity right hilum with associated segmental lesion; (?)carcinoma.

Course and treatment. Pitch warts underwent spontaneous regression. Other lesions excised and sites still kept under continued surveillance.

Comment. Pitch is the residue distillate of crude coal tar. Cancers in pitch workers were described by Legge (1910) and others. The premalignant skin changes in pitch workers are similar to chronic tar dermatosis (Fisher, 1965) of gas workers, having texture changes (hyperplasia or atrophy), pigmentation anomalies (melanosis and freckles from photosensitivity), vascular changes (telangiectasia), and hyperplasias (squamous keratosis, acanthomas, carcinomas and acneiform lesions).

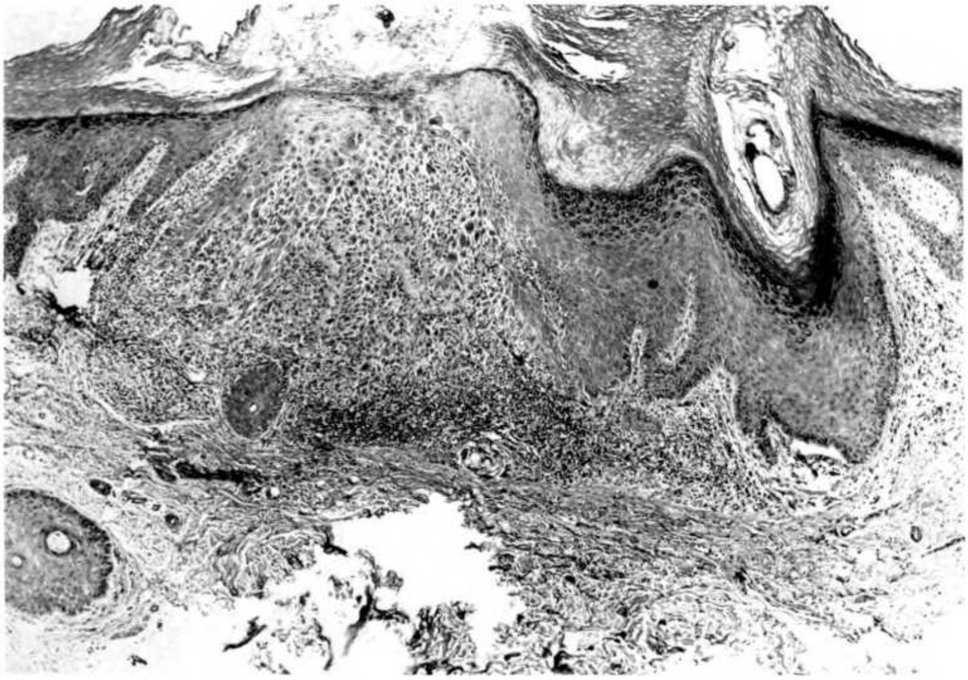

FIGURE 1. Pitch worker's skin.

Pitch warts are indistinguishable from keratoacanthoma and usually recede spontaneously. There is a direct relationship between the degree of exposure to pitch and neoplasia (pitch warts and squamous carcinoma), which is most likely to develop in those 45–60 years old. In this factory there is evidence of personal susceptibility, since only 10·4% of the working population developed 'warts' during a 3 year survey, and most of these had multiple lesions (Hodgson & Whiteley, 1970). The lung cancer is thought to be referable to smoking.

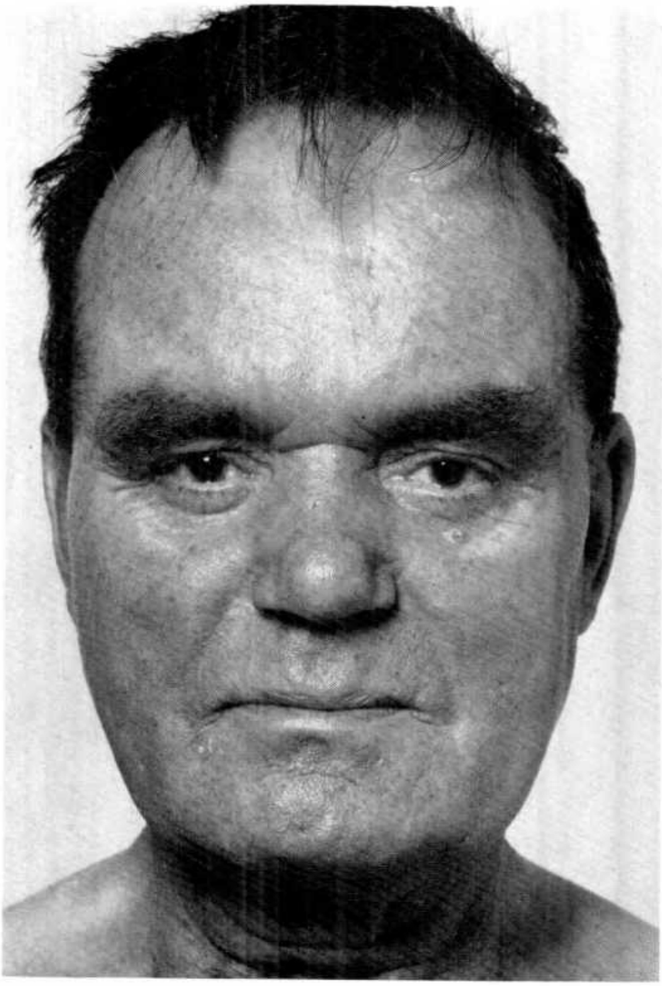

FIGURE 2. Squamous cell carcinoma of face in pitch worker (× 74).

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(29) Kyrle's disease. Hyperkeratosis follicularis et parafollicularis in cutem penetrans

DR C. LONG

Department of Dermatology, University Hospital of Wales, Cardiff

Mrs L.J., female, aged 60 years.

History. (1948). 'Rough papules' were noticed on both calves. Similar lesions developed on the thighs and arms over the next few years, and gradually became more numerous and larger. She now has horny plaques varying from 1 to 8 mm in diameter mainly on the lower legs (Fig. 1), but also on the thighs, forearms and arms. Very tiny crusts on crest of right pinna (Fig. 2). Some plugs were removed, leaving deep depressions. There were no warty or psoriasiform lesions to suggest hyperkeratosis lenticularis perstans (Flegel, 1958).

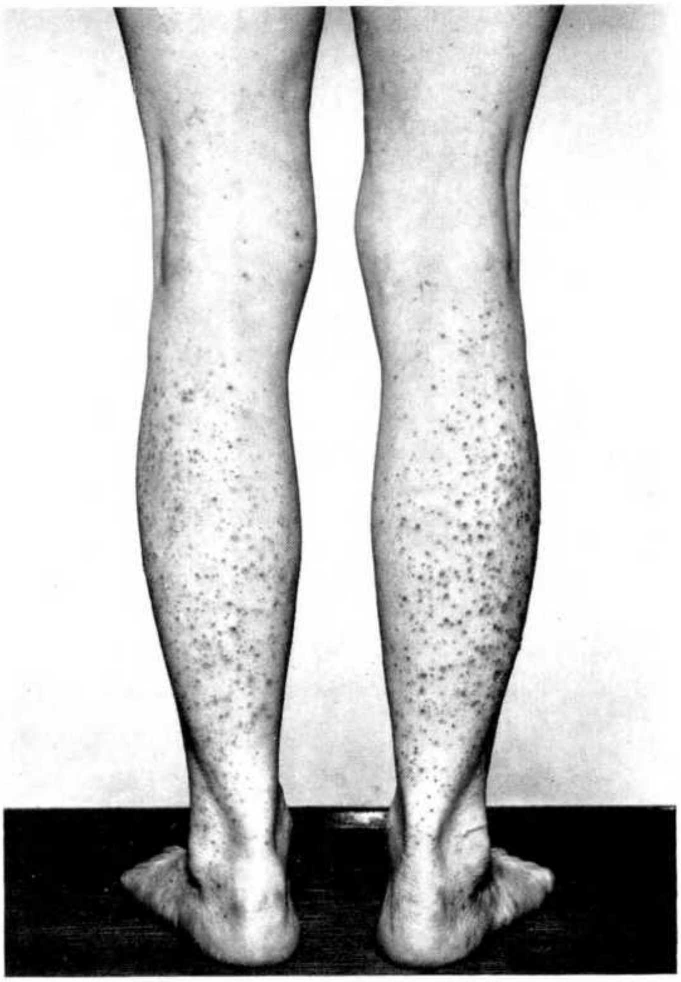

FIGURE 1. Horny plugs on legs.

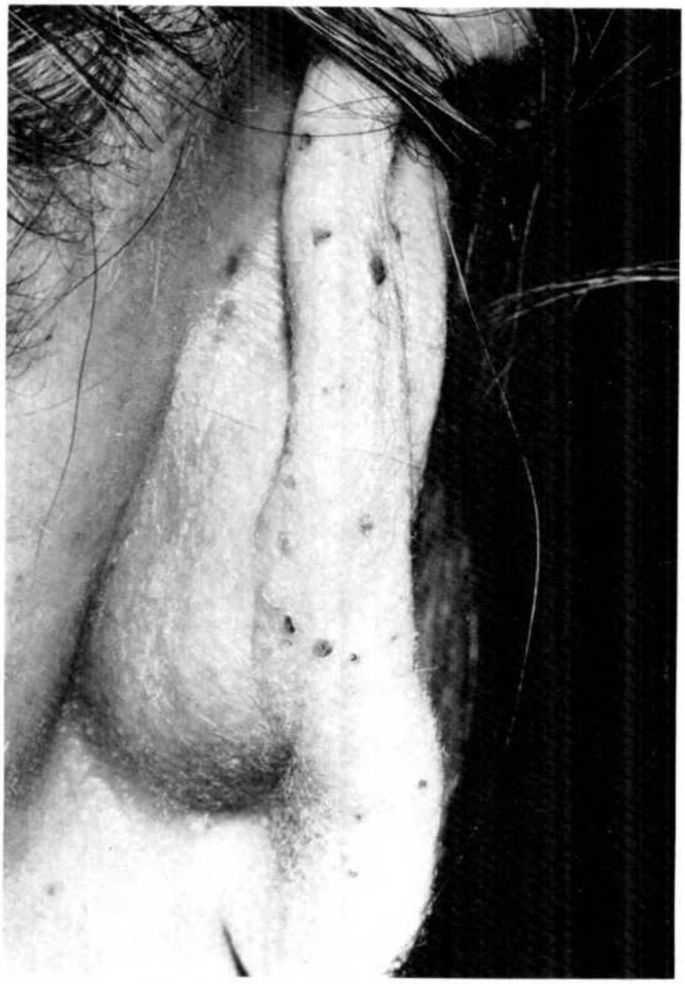

FIGURE 2. Horny plugs on pinnae.

Investigations. Histology. There is a localized hyperkeratosis, extending into the dermis as a laminated keratin plug. There is still squamous epithelium around the plug, with thinning of the stratum Malphigii and flattening of the rete ridges at the base of the lesion. There is round-cell infiltration of the upper dermis (Fig. 3).

Blood count: Hb, WBC, differential, ESR, normal. Urine, normal. Glucose tolerance test, normal. Liver function tests, normal.

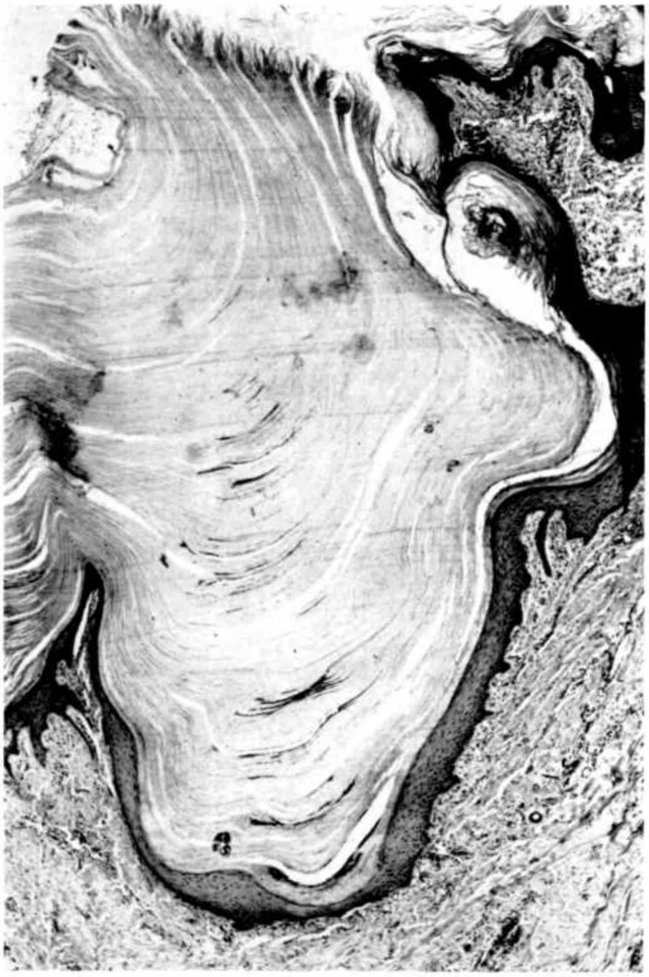

FIGURE 3. Section of horny plug on leg (× 33).

Treatment. Vitamin A 200,000 units daily for 8 months—no response. The cautery gave a good result with some lesions on both calves, but was too slow and painful.

Comment. In contrast with the findings of Carter & Constantine (1968), the glucose tolerance tests were normal.

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(30) Tuberous sclerosis

Dr C. Long

Department of Dermatology, University Hospital of Wales, Cardiff

T.L., male, aged 46 years.

History. Epileptic fits occurred between the ages of 8 and 27 years. Papules developed on the face at about 10 years of age and gradually enlarged. When he was first seen in the Dermatology Department in February, 1971, he had multiple lesions of adenoma sebaceum on the face, up to 8 mm in diameter around the alae nasi (Fig. 1). The lesions were removed with the cautery with marked cosmetic benefit. He also had fibromata on the gums, and under finger and toe nail folds (Fig. 2). He had a hagreen patch on the right flank, and a right internal strabismus which had been present for as long

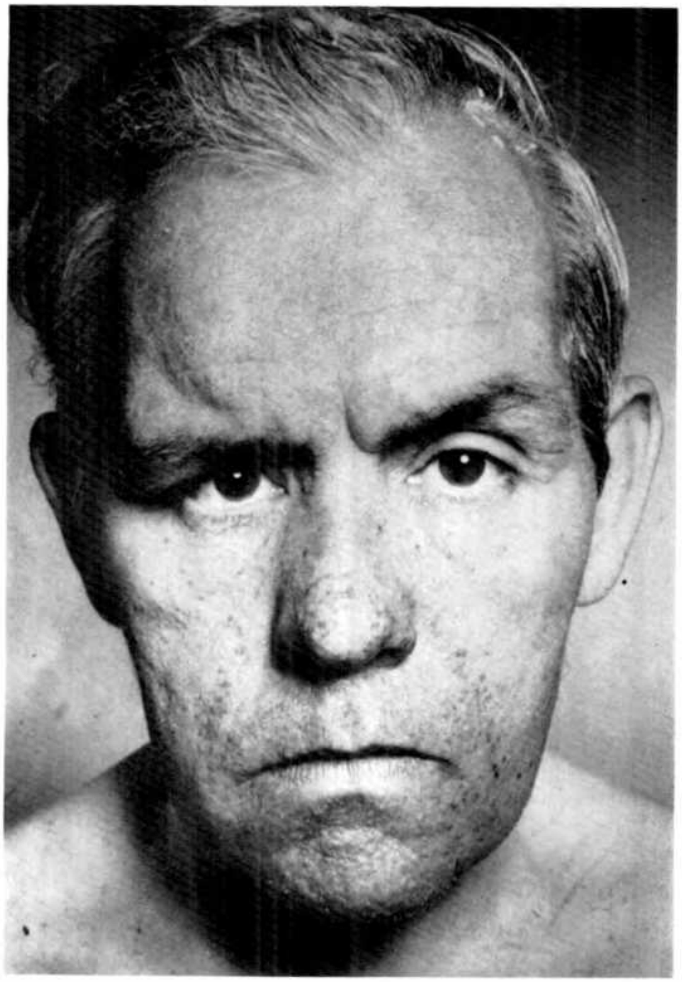

FIGURE 1. Adenoma sebaceum of face.

as he can remember. His IQ is low, but he works quite well as a factory labourer. There is no family history of skin or mucous membrane lesions or of mental retardation.

Investigations. X-ray skull—calcification of pineal body. X-ray chest, MSU, blood urea, EEG, ECG, all normal.

Comment. An interesting point is that the patient's 'epileptiform' attacks lasted only from the age of 8 to 27 years. His IQ seems good for such a case. X-ray of his skull shows only calcification of the pineal body. These findings conform to the suggestion that the less the tendency to form intracranial calcification the less is the brain damage (Dixon, 1945; Budenz, 1950). His grotesque adenoma sebaceum of the face did not recur after removal with cautery early in 1971.

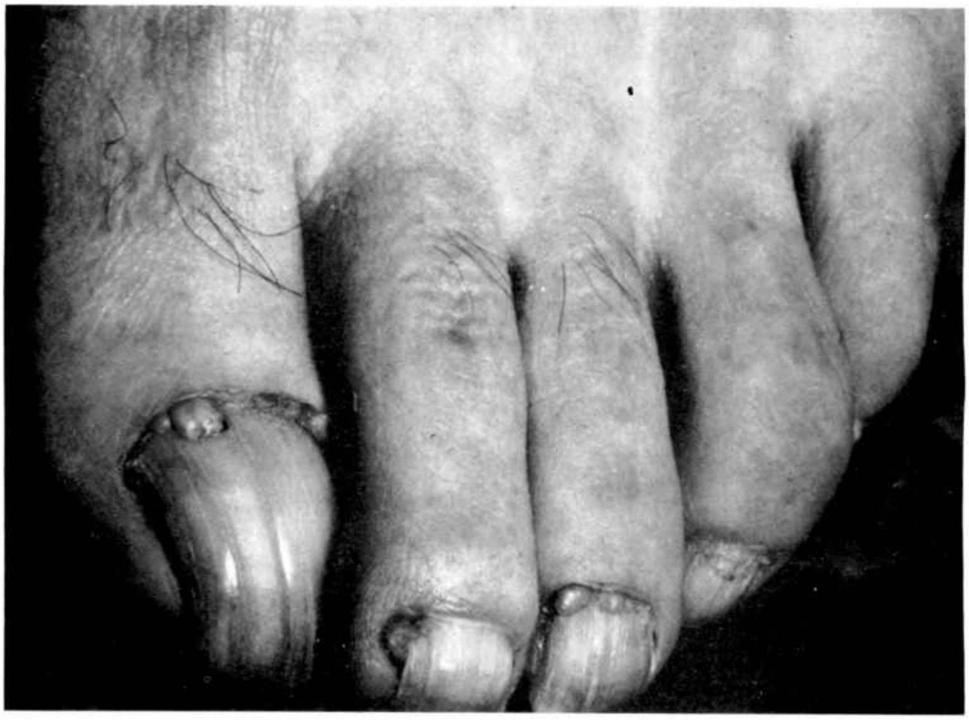

FIGURE 2. Fibromata under nail folds.

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(31) Yellow nail syndrome

DR G.C.MUSTON AND DR E.WADDINGTON

Department of Dermatology, University Hospital of Wales, Cardiff

H.E.M., male, aged 33 years.

History. 18 months ago this 33-year-old dairy checker noted that his nails were growing less rapidly, and needed cutting only once in 6–8 weeks. They had become yellowish-green in colour, and were more curved from side to side than formerly. 1 week before an emergency admission in October, 1971, he developed swelling and redness of the right leg, which was associated with a fever. He has never had any chest symptoms.

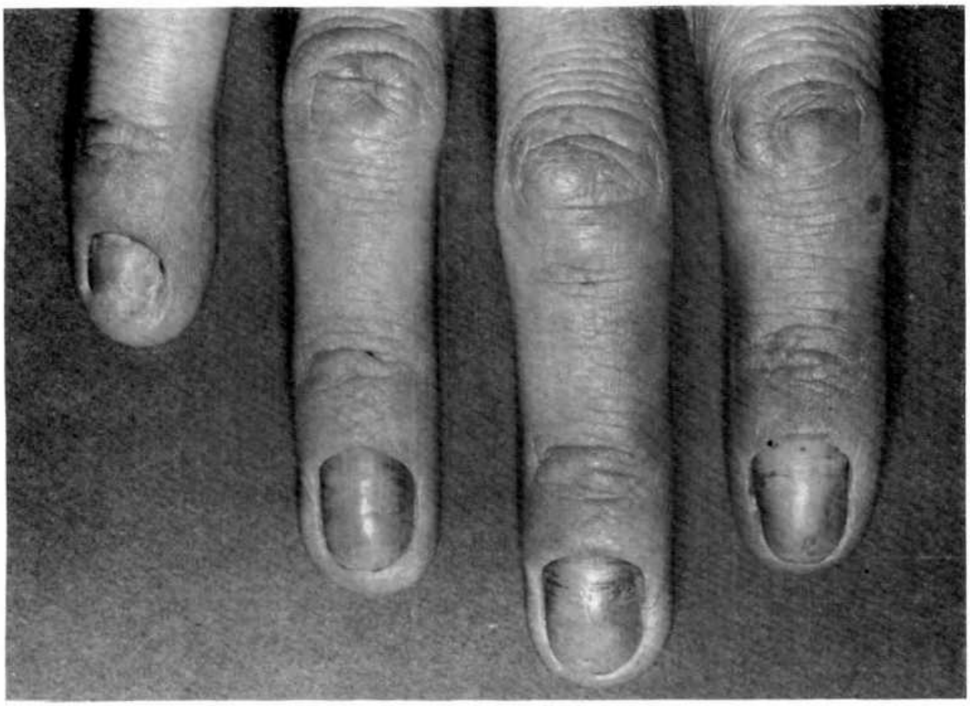

FIGURE 1. Nails showing absent cuticles, excessive curvature on their long axis, and discolouration of their edges.

Examination (Figs. 1 and 2). The nails of the hands and feet were yellowish-green in colour. The edges had a darker hue. The nails were smooth and thickened with excessive curvature on their long axis. The cuticle was deficient. There was lymphangitis with lymphoedema from the toe to the knee of the right leg. There was maceration of the skin between the fourth and fifth toes on both feet.

Investigations. A lymphangiogram using disulphine blue and lipiodol showed bilateral deficiency of the lymphatic channels, more marked on the right side (Fig. 3). Chest X-ray normal. Immunoglobulin electrophoresis normal. Skin scrapings; no fungus cultured.

Treatment. He was treated with systemic ampicillin and locally with Castellani's paint. The leg was elevated, and he wore an Elastoweb bandage when up and about. He has had no further episodes of lymphangitis.

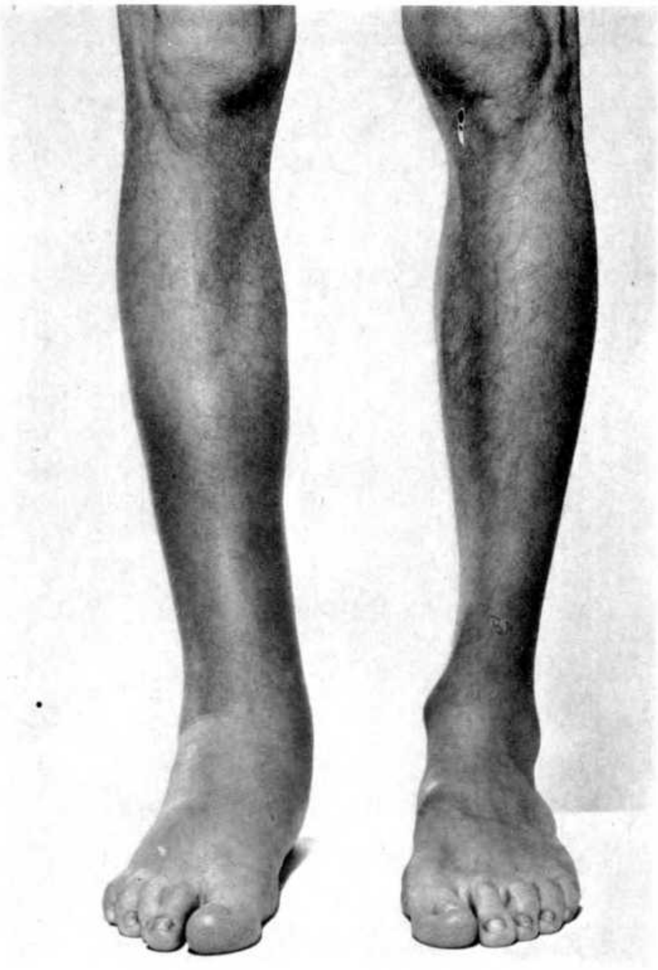

FIGURE 2. Lymphoedema of the right leg.

Comment. Since Samman & White (1964) first described the association of yellow nails and lymphoedema, Emerson (1966) and Miller, Rosenow & Osten (1972) have drawn attention to the importance of a careful search for respiratory changes in patients with yellow nails; in particular pleural effusions and bronchiectasis. Five of the twelve patients studied by Miller *et al.* (1972) had bronchiectasis. They suggest that in follow-up one might expect to see the emergence of a neoplasm of the lymphomatous or sarcomatous type.

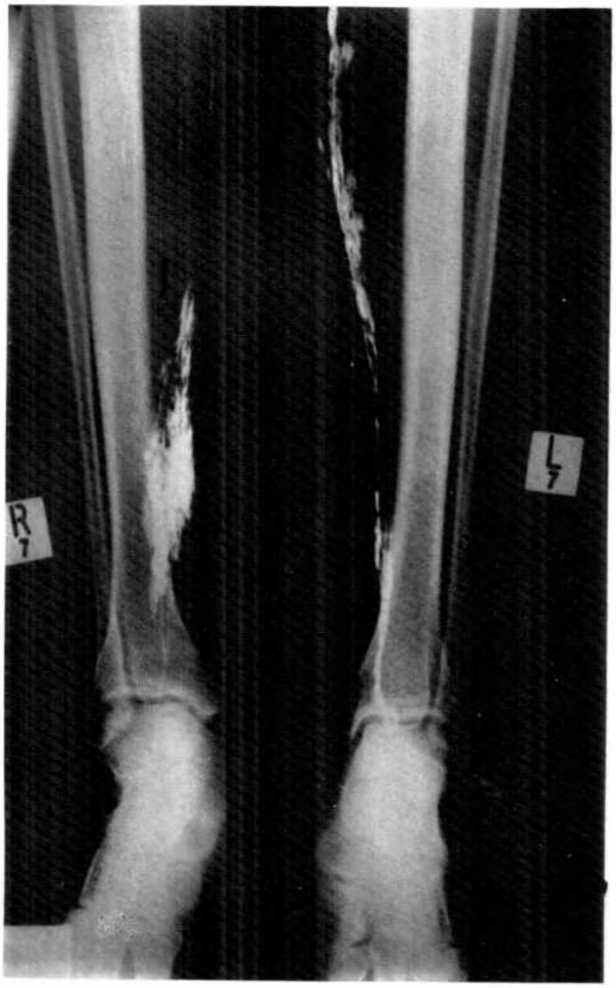

FIGURE 3. Lymphangiogram. Deficiency of lymphatic channels most marked on the right leg.

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